Methods for Assessing Biological Integrity of Surface Waters in Kentucky

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METHODS FOR ASSESSING BIOLOGICAL INTEGRITY OF SURFACE WATERS

Kentucky Department for Environmental Protection

Division of Water

Ecological Support Section

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This report has been approved for release:

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1 - INTRODUCTION

This manual has been developed by the Ecological Support Section of the Water Quality Branch (WQB) as guidance for the uniform collection, analysis, interpretation and quality assurance/quality control of biological samples from surface waters. The procedures defined herein are followed for all biological monitoring and assessment studies conducted by WQB and are recommended for use in assessments required by other Kentucky Division of Water (KDOW) programs. Compliance with these procedures is critical to basing sound assessments on waterbodies throughout the Commonwealth. Specific uses for these data are: (1) to determine legitimate waterbody uses as defined under Kentucky Water Quality Standards (KSWS) 401 KAR 5:031, (2) to determine effects of known point or nonpoint sources of pollution on the aquatic biota of the waterbody and (3) to determine background conditions within particular drainages or ecological regions.

Biological integrity is defined as "the condition of the aquatic community occurring in natural habitats of unimpaired surface waters as measured by community structure and function." *Biological assessments*, or bioassessments, are performed to evaluate the biological condition of surface water using biological surveys and other direct measurements of resident biota. The United States Environmental Protection Agency (U.S. EPA) document, "Biological Criteria: National program guidance for surface waters" (1990), has defined these and several other terms relating to biological assessments, and their definitions have been adopted by KDOW. Definitions for chapter-specific terms are provided in each chapter, where applicable.

The WQB integrates the collection and analysis of algal, macroinvertebrate, fish, habitat, bacteriological, and water chemistry data to arrive at conclusions on the health of Kentucky's surface waters. Because environmental stressors (e.g., excessive nutrients, organic wastes, industrial toxins and habitat alterations) reveal their impacts on the resident biota, biological communities leave detectable response signatures related to the various types of pollution. Therefore, environmental impacts and pollution abatement success can be directly measured with the standard ecological methods described in this manual.

As with any type of field sampling, safety precautions must be followed. Sampling during unsafe weather conditions (e.g., high water, electrical storms, extreme cold) should be avoided at all times. Field crews must consist of two or more people and all permanent employee crewmembers must be trained in First Aid and CPR. When using any field equipment (e.g. electrofishers, dredges, etc.), manufacturers' safety guidelines must be followed. Electrofishing units require extreme caution and all samplers must be trained in their use. To prevent injuries in the laboratory, safety procedures must be followed according to OSHA Standards.

If you have any questions or comments concerning this manual, please contact the Ecological Support Section at the following address:

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2 - STUDY PLANS AND STATION SELECTION

I. STUDY PLAN

The purpose of the study plan is to clearly state the objectives of the ecological survey, describe the study area, list known impacts to and uses of the waterbody, and list the types of data to be collected and measured. All available historical data are reviewed during design of the survey. The following sections are included in the study plan:

A. Waterbody Data

The following descriptive information is listed at the beginning of the study plan:
Stream name
Major river basin
Stream order (at mouth)
County or counties included in the survey
USGS 7.5-min. quadrangle names

B. Survey Dates

Tentative starting dates for the survey is listed on the study plan. For routine watershed monitoring (e.g., waterbody use-support classifications) appropriate sample index periods are stated in the algal, macroinvertebrate and fish chapters. These dates may not correspond to special situations (e.g., permitting activities and emergency spill or impact studies). Weather, streamflows or workload may affect actual starting dates. Surveys that are to be repeated seasonally are identified as such in the study plan.

C. Objectives

Biological surveys will have one or more stated objectives. Examples of objectives for biological surveys include, but are not limited to:

1. Determining legitimate waterbody uses as defined under Kentucky Surface Water Standards (KSWS) 401 KAR 5:031.
2. Determining effects of known point or nonpoint sources of pollution on the aquatic biota of the waterbody.
3. Determining background conditions within the drainage.

D. Study Area Description

The study area is described in detail and includes the following information:

1. Physical description of the study area, including topography, geology, physiographic region and ecoregion;
2. Stream length;

3. Drainage basin area;
4. Major tributaries;
5. Flow characteristics based on existing or extrapolated data;
6. Land use (agriculture, mining, silviculture, urban, etc.);
7. Location of known point or nonpoint sources of pollution.

E. Parameter Coverage

Parameter coverage varies depending on the objective(s) of the survey. Full coverage includes collection of habitat, biological, physicochemical and sediment samples. Biological samples include algal, macroinvertebrate and fish collections. Sampling of more than one taxonomic group encompasses more than one trophic level (primary producers and secondary and tertiary consumers) and provides a more realistic evaluation of the aquatic ecosystem. Full coverage may also include fish/shellfish contaminant analysis, bacteriological analysis and/or toxicity testing. Analytical chemistry parameters are selected based on the objectives of the study. At a minimum, hand-held multi-parameter meters that measure temperature, dissolved oxygen, pH and specific conductance should be employed on each sampling occasion. Habitat assessment (Chapter 6) is based on conditions observed at a site and the immediate area around the site at the time of the survey.

F. Quality Assurance/Quality Control (QA/QC) Statement

The QA/QC statement describes the procedures used to ensure the completeness and accuracy of all data. Procedures for field and laboratory QA/QC are described in detail in the study plan if they differ from the routine QA/QC procedures outlined in this manual. Quality Assurance/Quality Control procedures will be discussed in each chapter of this document, where applicable.

II. STATION SELECTION

Stream sites are initially selected from 7.5 min. USGS topographical maps and/or Arcview GIS software. A reconnaissance visit, if necessary, is made to finalize the site selections. A sampling site should include habitats that are typical for the stream reach under study. Locally modified sites with channelized areas, impounded sections, etc., are avoided unless they are the focus of the study. In addition, sampling at or near the mouths of tributaries should be avoided if possible.

The Probabilistic (random) Sampling Program selects stations using a different method. The random survey approach is used to assess aquatic life use support for streams in each watershed management unit. For each basin management unit U.S. EPA in Corvallis, Oregon, is contacted to provide the population of streams to be assessed. This population consists of wadeable streams, orders 1^{st}-4^{th}. Fifty to 75 streams are typically assessed in each basin-year. All streams in the defined population are then randomly selected, along with the latitude and longitude of the exact location where the assessment is to be conducted. The stream population is weighted so the less numerous, higher order (3^{rd} and 4^{th}) streams will have an equivalent chance of being selected, based on percentages of each stream order in a given basin. Often these sample sites have no public access and landowner permission to gain access must be obtained.

A. Number and Location of Sampling Sites

The number of sites selected for a survey often depends on the size of the drainage basin and the severity and number of impacts to the stream. However, available personnel and workload, funding or timeline may also limit the magnitude and scope of the study. All point sources, nonpoint sources and inputs from major tributaries are bracketed with an upstream site, a site at the mixing zone of each pollution source and intermediate sites. Control and reference sites (see below) and downstream recovery sites are also established. Downstream sites are selected between the point source and the next major tributary to avoid dilution effects. The number of downstream sites, and distance between these sites, depends on the objectives of the survey, the number of pollution sources, the number of major tributaries and the types of pollutants entering the stream. When the rate of movement of a contaminant or effluent plume is required, several samples are collected along a longitudinal gradient at regular intervals based on flow and travel time of the stream.

B. Control/Reference Sites

Whenever possible, at least two control sites are sampled for comparative purposes. Control sites are located either upstream of the source(s) of pollution or, when this is not possible, on a nearby, unaffected tributary. Streams within the same drainage basin and ecoregion and with similar physical characteristics and habitats should be used. Control sites from different drainage basins should be used only if no suitable site can be located within the basin of the stream being surveyed. Fixed ecoregional reference sites (streams that are considered the most natural and undisturbed for the particular ecoregion) should be used where possible.

C. Tributary Sites

When applicable, sites may be selected on each major tributary. Upstream and downstream sites to determine tributary and dilution effects should bracket the tributary.

D. Recovery Sites

At least one site should be located far enough downstream of the polluted area to detect recovery of the aquatic biota (i.e., when the biota begins to show a similarity to the control/reference site). Location of this site depends on the magnitude and downstream extent of the pollution.

E. Site Numbering

Stations are numbered consecutively from the mouth of the stream to the headwaters in a hierarchical method. The station nearest the mouth is assigned the lowest number not previously used. Sites are then numbered consecutively from mouth to headwaters. Next, tributary sites are assigned numbers, starting with the tributary closest to the mouth of the stream being surveyed. All stations on that tributary are numbered consecutively upstream from the confluence. Then, the next tributary is numbered until all sites have been assigned numbers. If additional sites are added after the initial study, they must be assigned numbers that have not been previously used, following the above outlined convention as closely as possible.

3 - ECOREGIONS AND THE REFERENCE CONDITION

I. INTRODUCTION TO ECOREGIONS

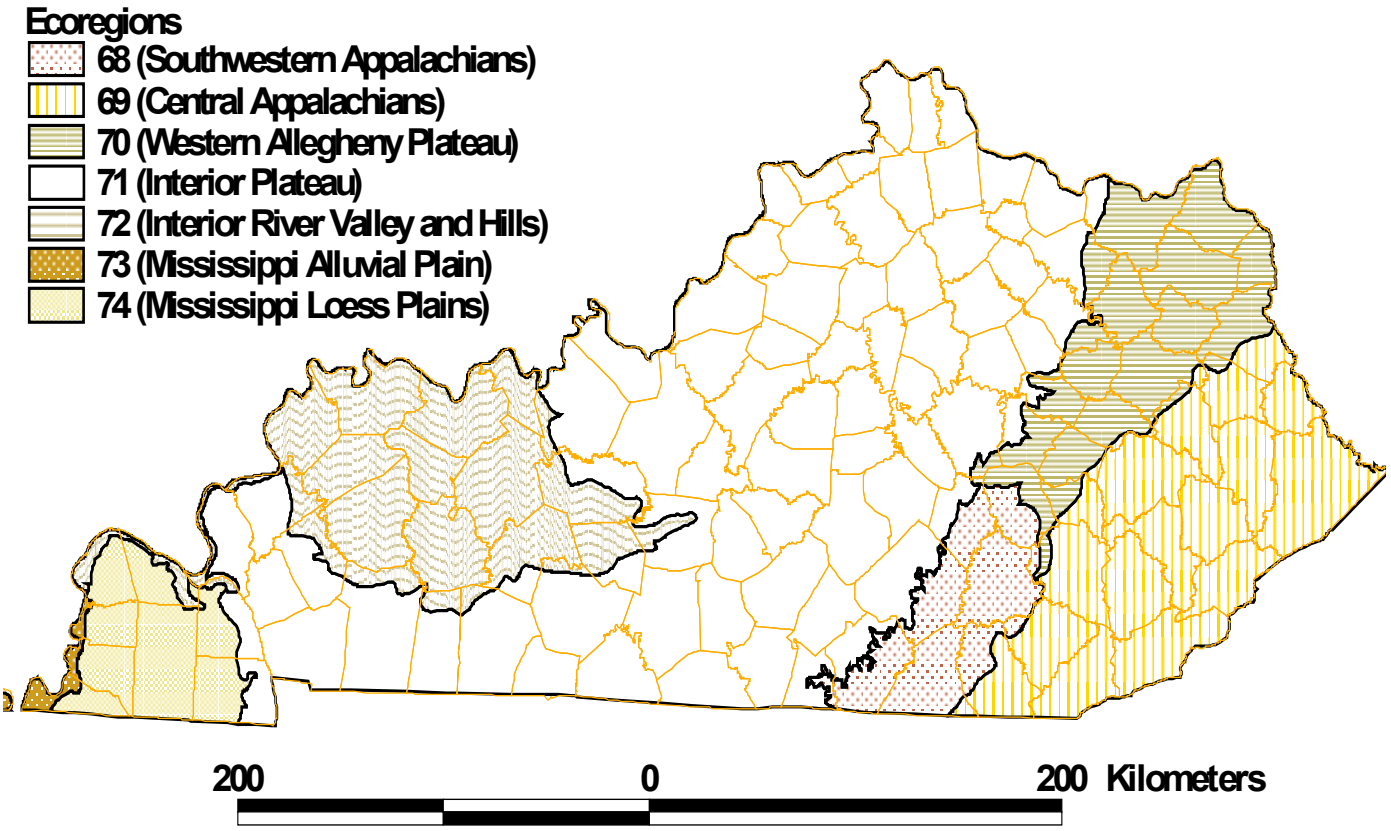
The concept of ecological regions, or ecoregions, within physiographic regions is a recent postulation that has received considerable attention. The conceptual framework of aquatic ecoregions is based on definable similarities among streams in a region that are related to the terrestrial characteristics of that region. In effect, streams acquire their characteristics from their watersheds (Likens and Bormann 1974, Hynes 1975). Streams draining watersheds of the same size, with comparable land uses and habitats, and in the same region are more likely to contain similar aquatic communities than those draining watersheds of a different size or in a different region (Hughes et al. 1986). This regional framework facilitates water quality resource management and the development of region-specific biological and chemical criteria.

Omernik (1987) mapped the Level III terrestrial ecoregions of the conterminous United States based on physiography, soil types, potential natural vegetation, geology and land-use types. A revised version of the Level III ecoregions in Kentucky is shown in Figure 3-1. Analysis of the data revealed regional homogeneities, which allowed for the delineation of boundaries between ecoregions and the location of the most typical areas within each ecoregion. Each of the delineated Level III ecoregions is descriptively compared below to adjacent ecoregions (modified after Woods et al. 2002). Level IV subecoregions have been delineated and a map and descriptions are available in Woods et al. (2002).

Ecoregion 68: SOUTHWESTERN APPALACHIANS

This region has a mixture of open, low mountains containing a mosaic of forest and woodland with some cropland and pasture. A deeply incised escarpment occurs in the west near the boundary with the Interior Plateau's Eastern Highland Rim (Ecoregion 71). The landscape is underlain by Pennsylvanian and Mississippian rock strata. Ultisols and Inceptisols (soil types) are common and contrast with the Alfisols that dominate the lowlands of the Interior Plateau to the west. Mixed mesophytic forest is generally restricted to the deeper ravines and escarpment slopes, and mixed oaks with shortleaf pine dominate the upland forests. Resource extraction (oil, gas and coal), agriculture and silviculture are the major land uses. Moderate- to high-gradient streams are common and generally have cobble- or boulder-dominated substrates; a few low-gradient streams occur and have gravelly or sandy bottoms. Nutrient and alkalinity levels are typically low compared to adjacent Ecoregion 71. Fish and mussel distributions in Cumberland River tributaries are distinct from the tributaries of the Kentucky River. They are also distinct from areas above Cumberland Falls in Ecoregion 69.

Figure 3-1. Level III Ecoregions of Kentucky



Ecoregion 69: CENTRAL APPLACHIANS

Ecoregion 69 is a high, dissected and rugged plateau made up of sandstone, shale, conglomerate and coal of Pennsylvanian age. The plateau is locally punctuated by a few anticlinal ridges. Its rugged terrain, cool climate and nutrient-poor soils sharply limit agricultural potential and result in a mostly forested land cover. The high hills and low mountains are mostly covered by mixed mesophytic forest. Bituminous coal mines are common and have caused the siltation and acidification of streams. Soils have developed from residuum and are mostly Ultisols and Inceptisols; they contrast with the Alfisols that dominate the lowlands of the Interior Plateau (Ecoregion 71) to the west. Its western boundary with Ecoregion 70 occurs at the elevation and forest density break; the more densely forested Ecoregion 69 is higher, cooler, and steeper than the Western Allegheny Plateau (Ecoregion 70) and is underlain by more resistant rock. The region is drained by relatively high gradient streams that make up the headwaters of the Kentucky, Cumberland, Licking and Big Sandy river basins. These streams are typically cool, with substrates composed mainly of cobbles and boulders.

Ecoregion 70: WESTERN ALLEGHENY PLATEAU

The hilly and wooded terrain of Ecoregion 70 was not muted by glaciation. It is more rugged than the agricultural till plains of Ecoregions 55 and 61 in Ohio, but is less rugged than the Central Appalachians (Ecoregion 69). Extensive mixed mesophytic forests originally grew in Ecoregion 70, and contrast with the oak-hickory forest that is found farther west in the lower Interior Plateau (Ecoregion 71). Today, most of the rounded hills of Ecoregion 70 remain in forest; dairy, livestock and general farms, as well as residential developments, are concentrated in the valleys. Resource extraction (oil, gas and coal), silviculture and agriculture remain the major land uses within this ecoregion. Horizontally bedded, Pennsylvanian sedimentary rock containing sandstone, siltstone, shales and coal underlies the region. Some areas have eroded down to limestone and may have localized karst development. Moderate to high-gradient headwater streams of the Kentucky, Licking, Little Sandy and Ohio river basins originate within the Western Allegheny Ecoregion. Upland streams are generally cool with cobble-boulder substrates similar to Ecoregion 69. Nutrient and alkalinity levels are generally higher than the Central Appalachians (Ecoregion 69) but lower than the Interior Plateau (Ecoregion 71).

Ecoregion 71: Interior Plateau

The Interior Plateau Ecoregion is the largest ecoregion in area encompassing most of the Highland Rim and Blue Grass Sections of the Interior Low Plateau Physiographic Province. Ecoregion 71 is composed of irregular plains, open hills, knobs and large areas of karst topography. Its landforms and soils are the result of the differential weathering of sedimentary strata, stream erosion and deposition, and the solvent action of subterranean water on carbonates. Ecoregion 71 is underlain by Mississippian-through Ordovician-age limestone, chert, sandstone, siltstone and shale. Lithologic and related physiographic boundaries tend to determine the natural subdivisions of the Interior Plateau (71). In areas underlain by cavernous limestone, drainage is primarily underground and only a few entrenched master streams and rivers occur. Rock types are distinct from the unconsolidated coastal plain sands that underlie the Mississippi Valley Loess Plains (74) in western Kentucky and the Pennsylvanian coal measures found in Ecoregions 69, 70 and 72. Maximum elevations and local relief are lower than in the Appalachian and Cumberland Plateau ecoregions to the east (68, 69 and 70) but are greater than in the Interior River Valley and Hills (72). The soils of Ecoregion 71 are highly varied and developed from the underlying sandstone, siltstone, shale and limestone and are not from glacial till like those of the Eastern Corn Belt Plains (55) to the north. Lowlands are generally dominated by Alfisols and not Ultisols or Inceptisols that are more characteristic of` Ecoregions 68, 69 and 70. The natural vegetation is primarily oak-hickory forest, but large areas of bluestem prairie originally occurred in the western karst areas. The potential natural vegetation is distinct from the mixed mesophytic forests of higher, cooler, wetter Ecoregions 68, 69 and 70. Rolling pastures, crop fields and woodlots are common, and agriculture, urban expansion and construction are major land uses. Most of the streams in the Interior Plateau Ecoregion are heavily influenced by human activities. Parts of the Licking, Kentucky, Cumberland, Salt, Green, Tradewater and Ohio river basins are located within the ecoregion. Stream morphology is highly variable across this large ecoregion. Both moderate-to high-gradient streams with boulder-cobble substrates and low-gradient streams with sand-gravel bottoms occur. Cool groundwater-fed streams punctuate those areas underlain by extensive and well-developed karst geology. Stream nutrient, alkalinity and hardness levels are higher in most parts of Ecoregion 71 than in Ecoregions 68, 69 and 70.

Ecoregion 72. Interior River Valleys and Hills

This broad, undulating lowland was formed in nonresistant, noncalcareous sedimentary rock of Pennsylvanian age. Often referred to as the "Western Coalfields," it is rich in coal reserves. Large upland areas are veneered by windblown material. Many wide, flat-bottomed, terraced valleys occur and are filled with alluvium, loess and lacustrine deposits. Bottomland hardwood forests and swamp forests once grew on poorly drained, nearly level sites, whereas the upland areas had oak-hickory forests. Patterns of land use are more varied than in the neighboring ecoregions, and large areas have been strip mined for coal. Drained alluvial soils are farmed for feed grains and soybeans. Undrained valleys are used for forage crops, pasture or woodlots; uplands are used for mixed farming and livestock. Extensive strip mining, as well as crop and livestock production, have impacted stream water quality (acidification, sedimentation, nutrients) and stream habitat (channelization, sedimentation); sheet erosion can be severe on cultivated slopes. Streams feed the Tradewater, Green and Ohio river basins and have relatively low (<5 ft/mile) gradients. Unchannelized upland streams often have gravel bottoms, while sand, silt and mud dominate most lowland channels. Streams typically have lower nutrient, alkalinity and hardness levels than those in Ecoregion 71 do. Wetlands were once common in this region, but many have since been drained for agricultural uses.

Ecoregion 73: Mississippi Alluvial Plain

The Mississippi Alluvial Plain is the smallest ecoregion found in Kentucky, occupying the areas immediately adjacent to the Mississippi River. Rock stratum is almost exclusively composed of alluvial deposits. Mostly flat, broad floodplains with river terraces and levees provide the main elements of relief. Abandoned channels, oxbow lakes, bayous, backswamps and point bars are found in the ecoregion. Fine-grained, poorly drained soils are common, but better drained loamy and sandy soils also occur. Winters are milder and summers are hotter than other Kentucky ecoregions. Bottomland deciduous forest vegetation covered the region before clearance for cultivation. All of the streams within the area drain into the Mississippi River and have been extensively channelized for agriculture. Streams are very low-gradient and have sandy to muddy substrates and often contain extensive wetland vegetation.

Ecoregion 74: Mississippi Valley Loess Plains

Ecoregion 74 in far western Kentucky consists of irregular plains, gently rolling hills and, near the Mississippi River, bluffs. It is mostly covered by thick loess and alluvium and underlain by Cretaceous and Tertiary coastal plain sediments. Rock types are distinctly different from the limestone, chert, sandstone, siltstone and shale of the Interior Plateau (71). Ecoregion 74 has less relief than ecoregions farther to the east in Kentucky, and elevations are much lower than in the Appalachian ecoregions. The potential natural vegetation is oak-hickory forest (Kuchler 1966), but agriculture is the dominant land use in Ecoregion 74 in Kentucky. Typically, streams have moderate to low gradients and gravelly to sandy bottoms. Wetlands are common throughout the area. In natural streams, nutrient and alkalinity levels are generally low compared to those of Ecoregion 71.

II. DEFINING ECOREGIONAL REFERENCE CONDITIONS

To address levels of impact on any given stream, a firm understanding of the inherent biological variability and potential of natural streams in a collective region is necessary. This is accomplished using a regional reference approach (Hughes et al. 1986) which is based on the range of conditions found in a population of sites or streams with similar physical characteristics and minimal human impact. Reference sites are tightly associated with ecoregions and an effort is made to obtain good coverage of sites within all regions. Collectively, the reference condition refers to the range of quantifiable ecological elements (chemistry, habitat and biology) that are found in natural environments. In many regions of Kentucky, finding reference streams can be a difficult task, as few regions are without areas of human disturbance. Therefore, reference reaches are more appropriately deemed "least-disturbed." The application of the reference condition involves its comparison to a stream exposed to environmental stress using defined sampling methodology and assessment criteria. Impairment of the test site would be detected if indicator measurements (e.g., species richness, habitat rating, nutrients) fell outside the range of threshold criteria established by the reference condition.

1. Selection of Candidate Reference Reach Waterbodies

Ecoregional reference reach site selection and evaluation analysis is an ongoing process supported by intensive map and ground reconnaissance. The U.S. Environmental Protection Agency's (EPA) Biocriteria Program suggests that the selection process for candidate reference reach waterbodies should be well documented so that the data defining the reference condition will be scientifically defensible.

In order to comply with U.S. EPA guidelines, the Reference Reach Program follows a step-by-step process for the selection of candidate reference reach waterbodies. This process involves the analysis of topographic maps and aerial photography, cross-referencing with other available data sources and field reconnaissance of the candidate watersheds. Because of the diverse land use, topography and physiography across the state, fixed statewide reference criteria are not used. Therefore, reference reach candidates are qualified in a regional context in an effort to find "least-disturbed" conditions among the various ecoregions.

U.S. Geological Survey (USGS) 7.5-min. topographic maps and aerial Digital Orthogonal Quadrangles (DOQs) lying within the boundaries of the Commonwealth of Kentucky are analyzed as part of the initial step of the selection process. Each stream is then evaluated based upon the presence or absence of the following:

1) High proportion of forestland and riparian zone quality;
2) Towns or communities along the streambank;
3) Resource extraction in the watershed (e.g., coal mines, oil wells, gas wells);
4) Hydrologic modification in the watershed (e.g., impoundment, channelization);
5) Major sewage treatment/industrial discharges.

If an adequate riparian zone exists along most of the stream length and minimal land-use activities occur within the watershed, then the stream is recorded as an initial candidate. An initial candidates' list is then compiled for each county and Level III ecoregion. Once the initial

candidates' list is created, it is cross-referenced with other available data sources including the following:

1) Kentucky Rivers Assessment (KDOW and NPS 1992);
2) Kentucky Nonpoint Source Assessment Report (KDOW 1999);
3) Kentucky Nature Preserves Commission's Fish Collection Catalogue (Warren et al. 1983);
4) Kentucky Division of Water's Fish Collection Catalogue (Mills 1988);
5) Aquatic Biota and Water Quality Survey of the Appalachian Province, Vol. 1-3 (Harker et al. 1979);
6) Aquatic Biota and Water Quality Survey of the Upper Cumberland River Basin, Vol. 1-2 (Harker et al. 1980);
7) Aquatic Biota and Water Quality Survey of the Western Kentucky Coal Field, Vol. 1-2 (Harker et al. 1981);
8) Recommendations for Kentucky's Outstanding Resource Water Classifications with Water Quality Criteria for Protection (KNPC 1982);
9) Kentucky Division of Water Intensive Survey Reports (KDOW); and
10) Personal communication with Kentucky Division of Water, Kentucky Nature Preserves Commission, Kentucky Department of Fish and Wildlife Resources, and Daniel Boone National Forest personnel, where applicable.

Cross-referencing allows for amendment of the initial candidates' list resulting in a smaller, secondary list. Upon completion of cross-referencing, field reconnaissance of the watersheds is conducted. Each site is evaluated on categories and criteria shown in Table 3-1.

Table 3-1. Summary of physical criteria used in the Reference Reach selection process.

Category	Criterion
1) riparian zone condition	well-developed providing some canopy over the stream; presence of adequate aquatic habitats in the form of root mats, coarse woody debris and other allochthonous material
2) bank stability	at least moderately stable with only a few erodible areas within the sampling station
3) degree of sedimentation	the substrate is 25 percent or less embedded by fine sediment
4) suspended material	the water is relatively free from suspended solids during normal weather conditions
5) evidence of nutrient enrichment	the substrate is relatively free from extensive algal mats that could choke riffle habitats
6) conductivity	conductivity is not highly elevated above what naturally occurs (region-specific)
7) aquatic habitat availability	there is a 50 percent or greater mix of rubble, gravel, boulders, submerged logs, root mats, aquatic vegetation or other stable habitats available for aquatic organisms
8) the presence or absence of trash in the stream	solid waste within the stream and on the streambank is at a minimum
9) evidence of new land-use activities in the watershed	the land-use conditions remain constant from what is depicted on the most recent USGS topographic or DOQ maps
10) accessibility of the site for collection	Accessible

Note: the reference criteria listed above may vary somewhat among ecoregions.

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4 - SITE CHARACTERIZATION

I. INTRODUCTION

Assessing the quality of an area to be sampled is an integral part of any aquatic survey. In intensive impact surveys, every attempt is made to sample waterbodies, such as streams and wetlands, with comparable habitat types. Qualitative documentation of habitat quality is recorded on field data sheets. A more quantitative approach to determine the habitat conditions of a sampling site is the Habitat Assessment procedures found in Chapter 6.

II. FIELD DATA SHEET

Field observations of site conditions and the habitat assessment are recorded on a field data sheet (FDS), (Appendices A-1 and A-2, depending on high/moderate or low-gradient stream classification). This type of habitat analysis allows the investigator to quickly check off or record observed habitat conditions. Procedures for scoring habitat features with the EPA Rapid Bioassessment Protocol (RBP) Habitat Assessment are presented in Chapter 6.

A. General Information

Some of the information included on the FDS is gathered in advance, using data from maps and prior reconnaissance visits.

1. Map Information

The following FDS information is gathered from maps, primarily using USGS 7.5 minute topographical maps and county maps: basin, stream name, location (e.g. highway no., bridge, nearby town), county, latitude and longitude, map name or KDOW map number.

2. On-Site Information

The following general information is recorded on the FDS onsite, prior to biological sampling: weather (e.g. hot, cold, rainy, dry, sunny, etc.), time (beginning) and sample type (e.g., biological, bacteriological, physicochemical, sediment, tissue).

a. Water Quality Observations

A portable, multi-parameter water quality meter that measures, at a minimum, pH, dissolved oxygen, conductivity and water temperature should be used on each sampling occasion and recorded on the FDS. In addition, note any water odors, water surface oils and water clarity/turbidity observations.

B. Stream Substrate Quality and Composition

1. Substrate Composition

In general, variations in particle size and type are reflected in flowing bodies of water by gradation of habitat types from stream headwaters to mouth. Each longitudinal gradation in substrate type harbors a characteristic biotic community. The absence of characteristic

community members in the presence of a favorable substrate type can be a useful indication of stream disturbance. For visual estimates of substrate size, a transect is surveyed in a pool (mid-pool) and riffle (mid-riffle) to estimate the substrate by percent particle size and type of material. Results are expressed as percent of total. Additionally, a Wohlman Pebble Count (or equivalent) can be conducted following procedures found in (Harrelson et al. 1994, Wolman 1965). Sample particles are measured against the particle size chart (Table 4-1) to provide the investigator with a fixed concept of category size. In deep waters, particle size may be determined from dredge grab samples. Results are recorded on the FDS. In addition, record the estimated percent riffle, run, and pool within the sampling reach. References: (Cummins 1962, Platts et al. 1983, Wentworth 1922, Winger 1981)

Table 4-1: Substrate Particle Size Chart

Categories	Size (mm)
Boulders	>256 (= 10 in)
Cobble	64 - 256 (= 2.5 - 10 in)
Pebble	16 - 64 (= .63 - 2.5 in)
Gravel	2 - 16 (=.08 - .63 in)
Fines	<2 (= .08 in)
Exposed Bedrock	--
Hardpan Clay	--
Detritus	--

C. Stream Physical Features

1. Stream Flow Condition

The condition of the stream is evaluated, and one of the following assessments is recorded on the FDS: perennial, intermittent or interrupted. In addition, record the gradient of the stream as either high, medium or low.

2. Stream Discharge/Velocity

The stage of the stream is estimated, and one of the following assessments is recorded on the FDS: dry, no flow (pooled), low, normal, high or flooded. Velocities are measured with an acceptable flow meter or with neutral-buoyant objects (e.g., oranges, small sticks, small sponge rubber balls) in areas of laminar flow and along a uniform transect of the channel. Reference: (Lazorchak, Klemm, and Peck 1998)

3. Channel Morphology

a. Stream-Depth Range

The stream-depth range is estimated for the entire reach and recorded on the FDS.

b. Stream-Width Range

The stream-width range is estimated for the entire reach and recorded on the FDS.

4. Canopy

An exposed stream often experiences increased water temperatures that may be directly or indirectly limiting to some organisms and may be favorable for nuisance algal blooms and decreased dissolved oxygen. Light intensity may be limiting to some organisms and favorable to others. A partially shaded stream generally achieves the greatest diversity. In wadeable streams, sufficient shade to maintain temperatures and habitats that will support indigenous organisms is generally created by a 50% to 75% tree canopy. Natural headwater streams should generally have 75% to 100% tree canopy.

The percent canopy shading the stream is recorded on the FDS using these categories.

Fully Exposed	(0 - 25%)
Partially Exposed	(25% - 50%)
Partially Shaded	(50% - 75%)
Fully Shaded	(75% - 100%)

References: (Hawkins et al. 1982, U.S. EPA 1982, Pfankuch 1978, Karr and Schlosser 1977, Winger 1981, Platts et al. 1983)

5. Channel Alterations

Many activities that alter the stream channel require water quality certification by KDOW and Section 404 permits from the U.S. Army Corps of Engineers. Some of these activities include: dredging, channelization, clear and snag, bridge construction and artificial bank stabilization. The occurrence of any channel-altering activities at the site is recorded in the FDS.

References: (Simpson et al. 1982, Johnson and McCormick 1978, Hewlett 1978, Newbold et al. 1980, Burns and Hewlett 1983, Platts 1978, 1981a, 1981b and 1982, Platts et al. 1983).

6. Hydraulic Structures

Hydraulic structures include any natural obstructions or human-made devices that impede or deflect the course of water from its original pathway. The presence and type of hydraulic structures are noted on the FDS. Some typical examples are dams, bridge abutments, islands, gravel and mud bars and any others to be listed individually.

7. Watershed Features/Land Use

All land uses occurring within the vicinity of the sampling site are recorded on the FDS. Examples of land use include silviculture, agriculture, oil exploration, construction, mining, urbanization, onsite wastewater treatment (e.g. "straight pipes"), etc. In addition, note the intensity of localized watershed erosion as heavy, moderate or none.

8. Pollution Types

The presence and proximity of point source discharges, such as municipal, private or industrial wastewater treatment plants (WWTP), should be noted on the FDS. In addition, record any localized nonpoint source impacts near or upstream of the sampling location.

D. Riparian Vegetation

Indicate the dominant trees, shrubs and herbaceous plants in the riparian zone. Because of its stabilizing effects and its ability to influence water temperatures, a riparian zone of 18 meters or more is preferred. The width of the riparian zone is scored in the RBP Habitat Assessment (Chapter 6). In addition, count the number of canopy strata present in the riparian zone as an indication of riparian age and quality (e.g., overstory, understory, herb layer).

References: (Hawkins et al. 1982, Schlosser and Karr 1981a and 1981b, Hewlett 1978, Karr and Schlosser 1977 and 1978, Johnson and McCormick 1978, Karr et al. 1981, Platts et al. 1983)

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5 - BACTERIOLOGICAL EVALUATION

I. INTRODUCTION

Bacteriological analysis includes, but is not necessarily limited to, the collection and analysis of *E. coli* and fecal coliform bacteria; i.e., bacteria that are commonly found in the intestinal tracts of warm-blooded animals.

II. BACTERIOLOGICAL SAMPLE COLLECTION

A. Samples are collected from directly below the water surface in sterile 250 ml Nalgene bottles, 125 ml borosilicate glass bottles containing sodium thiosulfate to counteract the presence of chlorine, or Corning sterile disposable 120 ml coliform water sample containers (1700-100), labeled, placed on wet ice and analyzed within eight hours of collection, preferably within six hours.

B. Stream samples are collected by using the surface grab technique and should be free of debris and bottom sediment. Grasp the bottle at the base with one hand and plunge the bottle, mouth down, into the water to avoid introducing surface scum. Position the mouth of the bottle upstream toward current. Collect the sample from a depth of 6-12 inches. Allow an air space for mixing in each sample (approximately 25%). In a 250 ml capacity sample bottle, collect approximately 200 ml. If more than one sample is taken, identify each sample with a number (i.e., 1, 2, 3) in chronological order of collection. This order should be repeated on the chain-of-custody sheet. If direct stream sampling is not practical, an alternative sampling device may be used (i.e., rod and reel, as long as sterile sampling bottles are used at each sample station).

C. A grab sample is obtained using a sterile sample bottle of 250 ml capacity if more than one bacterial parameter is to be analyzed. Identify the sampling site on a chain-of-custody tag and/or sheet and on a field log sheet, including location, time, date and the name of the actual sample collector.

D. Transport samples on wet ice in a cooler capable of holding a temperature of 1^{o}–4^{o}C.

E. Holding times for bacteriological samples should not be exceeded.

F. Note: Because of the reproduction potential of the bacteria, samples should be iced and analyzed as soon as possible, thus reducing the degree of error in the final result. Sewage samples and organically rich wastes are particularly susceptible to rapid increases or die-away; therefore, they should be held for the shortest time possible to minimize change.

1. If iced, samples must be delivered to the laboratory within six hours.
2. If not iced, samples must be delivered within one hour of collection, and processing must be initiated immediately upon delivery to avoid unpredictable changes. The time of collection and time of delivery to the laboratory must be documented, and proper chain-of-custody procedures followed.

Samples collected from a chlorinated source are dechlorinated by adding 0.1 ml of a 10% sodium thiosulfate solution per 125 ml of sample. This concentration will neutralize approximately 15 mg/l of residual chlorine.

III. SAMPLE HOLDING TIMES

A. A maximum time of eight hours may elapse from time of collection of the sample to completion of sample processing. Notify the laboratory at least 24 hours prior to sample collection, unless an emergency condition exists.

B. Once the samples have been processed, they must be resuscitated for two hours at 35 degrees Centigrade and incubated for 22 hours for *E. coli* (analyses by MTEC media procedure) or 24 (±2) hours at 44.5 degrees Centigrade for fecal coliform analyses. IDEXX procedure may take 48 hours. This means that samples delivered to the laboratory on Thursday or Friday require members of the staff to work Saturday or Sunday. Therefore, no samples will be accepted on Friday except in an emergency. No samples will be analyzed after normal working hours without prior approval. The laboratory must have time to prepare media, glassware and buffer solution for unexpected samples. The laboratory should be notified as soon as possible so that preparation can begin and conflicts can be avoided.

IV. GENERAL LABORATORY PRACTICE

The following analyses are performed in the bacteriological laboratory:

Total coliform analysis
E. coli analysis
Fecal coliform analysis

1. Total coliform analysis will be performed using IDEXX techniques and instrumentation (IDEXX Laboratories Inc., Westbrook, ME.)

2. *E. coli* procedures using membrane filter analysis will be performed as described in the latest edition of Standard Methods for the Examination of Water and Wastewater (Standard Methods).

3. Fecal coliform procedures using membrane filter analysis will be performed as described in the latest edition of Standard Methods.

4. Fecal coliform analyses for chlorinated effluents by membrane filter will be performed as described in the latest edition of Standard Methods.

5. For the purpose of resolving any controversy over results of membrane filter analysis, fecal coliform analyses will be performed, as described by the latest edition of Standard Methods, by the Most-Probable-Number Method.

6. A laboratory bench sheet provided in Appendix B-1 should be used to record findings.

References: APHA (1992), Weber (1973), U.S. EPA (1973)

V. INTERPRETATION OF BACTERIOLOGICAL ANALYSES

A. Total Coliform Analysis

This analysis will be performed during *E. coli* analysis with IDEXX (i.e., Quanti-Tray®/2000). It will provide information on water quality, but will not be used as a standard indicator. Total coliform analysis will be most beneficial in well water testing.

B. *E. coli* Analysis

Instream *E. coli* levels to determine water quality for primary contact recreational uses will be compared to maximum allowable limits established by U.S. EPA. Currently a single sample maximum of 235 per 100 ml is considered acceptable. *E. coli* testing by standard methodology (i.e., MTEC procedure) or IDEXX will be acceptable.

E. coli levels are used for:

1) primary contact recreation use determination
2) well water testing

C. Fecal Coliform Analysis

Instream fecal coliform levels will be compared to maximum allowable limits established in 401 KAR 5:031, Surface Water Standards.

Fecal coliform levels are used for:

1) primary contact recreation use determination
2) secondary contact recreation use determination
3) domestic water supply use determination
4) point/nonpoint assessment

VI. QUALITY ASSURANCE/QUALITY CONTROL

A. Duplication. Each laboratory must establish quality control over the microbiological analysis in use. Run duplicate analyses on one of every ten samples analyzed (10%) and a minimum of one duplicate analyses per month. The duplicates may be run as split samples by more than one analyst. At least once per month, two or more analysts should count the colonies on the same membranes containing 20 – 60 colonies.

B. Verification. Five percent of the samples measured should be verified as fecal coliform bacteria. Verify ten blue colonies as fecal coliform bacteria on one of every twenty samples. Lauryl tryptose broth and EC broth in 10 mL test tubes with gas fermentation vials made of borosilicate glass should be used for the analyses. Each analyst should verify ten colonies as fecal coliform bacteria a minimum of once per month.

C. Water Supply. Once per year a sample supply should be delivered and analyzed by the Division of Environmental Services for the presence of total organic carbon, ammonia nitrogen, cadmium, chromium, copper, lead, nickel, and zinc. Once per year test 100 ml of the water supply for the presence of bacteria.

D. Performance samples. Laboratories should analyze at least one unknown performance sample per year when available, for the parameters measured.

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6 - HABITAT ASSESSMENT

I. INTRODUCTION

A habitat assessment should be conducted at every biological sampling reach. Such an assessment will allow investigators to evaluate the quality of instream and riparian habitat. The availability of quality habitat directly influences the biological integrity of the stream reach. Information obtained from the habitat assessment can be used to supplement biological and physicochemical data when determining the overall health of the stream reach and stream-use designation. Additionally, habitat assessments can be used to document physical changes that occur at a sampling reach over time. In multi-agency monitoring projects (such as watershed monitoring), habitat assessments provide continuity and consistency between all entities involved in the monitoring effort. Habitat assessment procedures follow those outlined in *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers* (Barbour et al. 1999).

II. ASSESSMENT PROCEDURES

Investigators should conduct a visual-based habitat evaluation of the stream reach by filling out the appropriate Habitat Assessment Field Data Sheet (Barbour et al. 1999). In streams where riffles should naturally be present (e.g., most stream reaches of the Central Appalachian, Western Allegheny, Southwestern Appalachian and Interior Plateau ecoregions would qualify), the High-Gradient Habitat Assessment Field Data Sheet should be used (Appendix A-1). In low-gradient streams where rocky riffles are not naturally present (e.g., most stream reaches in the Mississippi Valley Loess Plain and the Interior River Lowland ecoregions would qualify), the Low-Gradient Habitat Assessment Field Data Sheet should be used (Appendix A-2).

The visual-based habitat evaluation consists of ten parameters that rate instream habitat, channel morphology, bank stability and riparian vegetation for each sampling reach. A numerical scale of 0 (lowest) to 20 (highest) is used to rate each parameter (Barbour et al. 1999). For each parameter, the investigators will determine which of the following conditions exist at the sampling reach: Optimal, Suboptimal, Marginal or Poor. A parameter score will then be given within the condition category chosen above: Optimal (20-16), Suboptimal (15-11), Marginal (10-6) or Poor (5-0). The investigators will total all of the parameter ratings to obtain a final habitat ranking (Barbour et al. 1999).

III. PARAMETERS FOR HABITAT ASSESSMENT

i. These parameters should be evaluated within the sampling reach. All of the areas within the reach should be evaluated together as a composite.

Parameter #1

Epifaunal Substrate/Available Cover (Both High and Low Gradient Sheets)- this metric measures the relative quantity and the variety of structures: cobble, boulders, fallen trees, logs, branches, root mats, undercut banks, aquatic vegetation, etc., that provide refugia, feeding opportunities and sites for spawning and nursery functions.

1. Optimal (High Gradient): >70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient) (20-16)

   Optimal (Low Gradient): >50% of substrate favorable for epifaunal colonization and fish cover, mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at a stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient) (20-16)

2. Suboptimal (High Gradient): 40%-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at the high end of the scale) (15-11)

   Suboptimal (Low Gradient): 30%-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of the scale) (15-11)

3. Marginal (High Gradient): 20%-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed (10-6)

   Marginal (Low Gradient): 10%-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed (10-6)

4. Poor (High Gradient): <20% stable habitat; lack of habitat is obvious; substrate unstable or lacking (5-0)

   Poor (Low Gradient): <10% stable habitat; lack of habitat is obvious; substrate unstable or lacking (5-0)

Parameter #2

1. **Embeddedness** - (**High Gradient Sheet) -** the extent to which rocks and snags are covered or sunken into the silt, sand, mud or biofilms (algal, fungal or bacterial mats) of the stream bottom. Generally, as rocks become embedded, the surface area available to macroinvertebrates and fish (for shelter, spawning and egg incubation) is decreased; assess in the upstream or central portions of riffles.

   a. Optimal: Rocks are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space (20-16)

   b. Suboptimal: Rocks are 25%-50% surrounded by fine sediment (15-11)

   c. Marginal: Rocks are 50%-75% surrounded by fine sediment (10-6)

d. Poor: Rocks are >75% surrounded by fine sediment (5-0)

2. **Pool Substrate Characterization** - (**Low Gradient Sheet)** - evaluates the type and condition of bottom substrates found in pools. Firmer sediment types (e.g., gravel and sand) and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock and no plants. In addition, a stream that has a uniform substrate in its pools will support far fewer types of organisms than a stream that has a variety of substrate types.

    a. Optimal: Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common (20-16)

    b. Suboptimal: Mixture of soft sand, mud or clay; mud may be dominant; some root mats and submerged vegetation present (15-11)

    c. Marginal: All mud or clay or sand bottom; little or no root mat; no submerged vegetation (10-6)

    d. Poor: Hard-pan clay or bedrock; no root mat or vegetation (5-0)

Parameter #3

1. **Velocity/Depth Regime** - (**High Gradient Sheet)** - the best streams in most high-gradient regions will have all of the following patterns of velocity and depth: 1) slow-deep, 2) slow-shallow, 3) fast-deep, and 4) fast-shallow; the occurrence of these four patterns relates to the stream's ability to provide and maintain a stable aquatic environment. Investigators may have to scale deep and shallow depending upon the stream size; a general guideline is 0.5 m between shallow and deep.

    a. Optimal: All 4 regimes present (20-16)

    b. Suboptimal: Only 3 of the 4 regimes present; if fast-shallow is missing, score lower than if missing other regimes (15-11)

    c. Marginal: Only 2 of the 4 regimes present; if fast-shallow or slow-shallow are missing, score low (10-6)

    d. Poor: Dominated by 1 regime (usually slow-deep) (5-0)

2. **Pool Variability** - (**Low Gradient Sheet)** - rates the overall mixture of pool types found in streams, according to size and depth. The four basic types of pools are large-shallow, large-deep, small-shallow and small-deep. A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitat to support a diverse aquatic community. General guidelines are any pool dimension (i.e., length, width, oblique)

greater than half the cross-section of the stream for separating large from small and 1 m depth separating shallow and deep.

a. Optimal: Even mix of large-shallow, large-deep, small-shallow and small-deep pools present (20-16)

b. Suboptimal: Majority of pools large-deep; very few shallow (15-11)

c. Marginal: Shallow pools much more prevalent than deep pools (10-6)

d. Poor: Majority of pools small-shallow or pools absent (5-0)

Parameter #4

Sediment Deposition (Both Sheets) - measures the amount of sediment that has accumulated in pools and changes that have occurred to the stream bottom as a result of deposition. This may cause the formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increases in size as the channel is diverted toward the outer bank) or shoals or result in the filling of runs and pools. Sediment is often found in areas that are obstructed and areas where the stream flow decreases, such as bends. Deposition is a symptom of an unstable and continually changing environment that becomes unsuitable for many organisms.

1. Optimal (High Gradient): Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition (20-16)

   Optimal (Low Gradient): Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition (20-16)

2. Suboptimal (High Gradient): Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5%-30% of the bottom affected; slight deposition in pools (15-11)

   Suboptimal (Low Gradient): Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20%-50% of the bottom affected; slight deposition in pools (15-11)

3. Marginal (High Gradient): Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30%-50% of the bottom affected; moderate sediment deposits apparent at most obstructions and slow areas, bends and pools (10-6)

   Marginal (Low Gradient): Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50%-80% of the bottom affected; sediment deposits at obstruction, constrictions and bends; moderate deposition of pools prevalent (10-6)

4. Poor (High Gradient): Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition (5-0)

Poor (Low Gradient): Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition (5-0)

Parameter #5

Channel Flow Status (Both Sheets) - the degree to which the channel is filled with water; will change with seasons.

1. Optimal: Water reaches base of both lower banks; minimal amount of channel substrate exposed (20-16)

2. Suboptimal: Water fills >75% of the available channel; or <25% of channel substrate exposed (15-11)

3. Marginal: Water fills 25%-75% of the available channel; riffle substrates are mostly exposed (10-6)

4. Poor: Very little water in channel; mostly present in pools (5-0)

ii. The next 5 parameters should evaluate an area from approx. 100-m upstream of the sampling reach through the sampling reach. This whole area should be evaluated as a composite. When determining left and right bank, look downstream.

Parameter #6

Channel Alteration (Both Sheets) - measures the large-scale changes in the shape of the stream channel; channel alteration is present when 1) artificial embankments, rip-rap and other forms of bank stabilization or structures are present, 2) the stream is very straight for significant distances, 3) dams and bridges are present and 4) other such changes have occurred.

1. Optimal: Channelization or dredging absent or minimal; stream with normal pattern (20-16)

2. Suboptimal: Some channelization present, usually in areas of bridge abutments; evidence of past channelization (dredging, etc., >20 past years) may be present, but recent channelization not present (15-11)

3. Marginal: Channelization may be extensive; embankments or shoring structures present on both banks; and 40%-80% of the stream reach channelized and disrupted (10-6)

4. Poor: Banks shored with gabion or cement; >80% of the stream disrupted; instream habitat greatly altered or removed entirely (5-0)

Parameter #7

1. **Frequency of Riffles (or Bends)** - (**High Gradient Sheet)** - measures the sequence of riffles and thus the heterogeneity occurring in a stream.

    a. Optimal: Occurrence of riffles relatively frequent; ratio of distance between riffles divided by the width of the stream <7:1 (generally 5 to 7); variety of habitat is key; in streams where riffles are continuous, placement of boulders or other large, natural obstruction is important (20-16)

    b. Suboptimal: Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 and 15 (15-11)

    c. Marginal: Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 and 25 (10-6)

    d. Poor: Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is >25 (5-0)

2. **Channel Sinuosity** - (**Low Gradient Sheet)** - evaluates the meandering or sinuosity of the stream. A high degree of sinuosity provides for diverse habitat and fauna, and the stream is better able to handle surges when water levels in the stream fluctuate as a result of storms. The absorption of this energy by bends protects the stream from excessive erosion and flooding and provides refugia for benthic invertebrates and fish during storm events. To gain an appreciation of this parameter in low-gradient streams, a longer segment or reach than that designated for sampling may be incorporated into the evaluation. In some situations, this parameter may be rated from viewing accurate topographical maps. The "sequencing" pattern of the stream morphology is important in rating this parameter. In "oxbow" streams of coastal areas and deltas, meanders are highly exaggerated and transient. Natural conditions in these streams are shifting channels and bends, and alteration is usually in the form of flow regulation and diversion. A stable channel is one that does not exhibit progressive changes in slope, shape or dimensions, although short-term variations may occur during floods (Gordon et al. 1992).

    a. Optimal: The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note-channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.) (20-16)

    b. Suboptimal: The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line (15-11)

    c. Marginal: The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line (10-6)

d. Poor: Channel straight; waterway has been channelized for a long distance (5-0)

Parameter #8

Bank Stability (Both Sheets) - measures whether the stream banks are eroded or have the potential to erode. Each bank is scored independently from 10-0.

1. Optimal: Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems; <5% of bank affected (10-9)

2. Suboptimal: Moderately stable; infrequent, small areas of erosion mostly healed over; 5%-30% of the bank affected (8-6)

3. Marginal: Moderately unstable; 30%-60% of bank in reach has areas of erosion; high erosion potential during floods (5-3)

4. Poor: Unstable; many raw, eroded areas; obvious bank sloughing; >60% of bank has erosional scars (2-0)

Parameter #9

Bank Vegetative Protection (Both Sheets) - measures the amount of vegetative protection afforded to the stream and the near-stream portion of the riparian zone. Each bank is scored independently from 10-0.

1. Optimal: >90% of the streambank surfaces and immediate riparian zones covered by natural vegetation, including trees, understory shrubs, herbs and nonwoody macrophytes; vegetation disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally (10-9)

2. Suboptimal: 70%-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one half of the potential plant stubble height remaining (8-6)

3. Marginal: 50%-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one half of the potential plant stubble height remaining (5-3)

4. Poor: <50% of the streambank surfaces covered by vegetation; disruption is very high; vegetation has been removed to 5 cm or less in average stubble height (2-0)

Parameter #10

Riparian Vegetative Zone Width (Both Sheets) - measures the width of the natural vegetation from the edge of the streambank through the riparian zone. The presence of old fields, paths, walkways, etc., in otherwise undisturbed riparian zones may be judged to be inconsequential to destruction of the riparian zone. Each bank is scored independently from 10-0.

1. Optimal: Width of riparian zone >18 m; human activities (parking lots, roadbeds, clear-cuts, lawns, pastures or crops) have not impacted the zone (10-9)

2. Suboptimal: Width of riparian zone 13-18 m; human activities have impacted the zone only minimally (8-6)

3. Marginal: Width of riparian zone 6-12 m; human activities have impacted the zone a great deal (5-3)

4. Poor: Width of riparian zone <6 m; little or no riparian zone due to human activities (2-0) (Barbour et al. 1999)

IV. TENTATIVE HABITAT CRITERIA

Habitat evaluations were conducted at biological sampling sites in the Kentucky River Basin for 1998, the Licking and Salt River Basins for 1999, the Cumberland, Tennessee and Mississippi River Basins for 2000, and the Green and Tradewater River Basins for 2001 using the new Rapid Bioassessment Protocols format. Historical and current reference reach habitat data were used to produce tentative habitat criteria for certain areas of Kentucky. The scores for all reference reach stations were ranked and divided into percentiles. The lower quartile (25^{th} percentile) may be considered the dividing line between those habitats fully supporting biotic integrity and those supporting biotic integrity, but threatened. Those scores falling within the 25^{th} and 10^{th} percentile may be considered supporting, but threatened. Habitats scoring in the last 10^{th} percentile may be considered partially supporting biotic integrity. Habitats may be considered poor if they fall below the lowest reference condition score for that area.

Mountainous ecoregions of the Commonwealth provided similar habitat opportunities for aquatic community colonization and use. Habitat scores from reference sites in the Central Appalachian, Southwestern Appalachian and Western Allegheny Ecoregions reflected these similarities. Therefore, habitat data from all of these ecoregions were combined to develop habitat criteria for the Mountains.

For the Interior Plateau Ecoregion, reference sites were divided into wadeable and headwater based upon drainage area. Those above six square miles were considered wadeable and below six square miles headwater. Headwater streams in this large ecoregion have a tendency to score higher on certain metrics (i.e. frequency of riffles, bank stability, riparian zone width) than wadeable streams as a result of increased land use. This bias was reflected in final habitat scores. Therefore, separate habitat criteria were developed for wadeable and headwater streams.

Streams sampled within the Mississippi Valley Loess Plains and Interior River Valleys and Hills Ecoregions were generally low-gradient streams with very few riffles, if any. Low-gradient habitat

assessment sheets were used in these ecoregions to rate the available habitat. Additionally, in low-gradient sections of Interior Plateau and mountainous streams, low-gradient habitat assessment sheets were used. Most low-gradient streams provide similar habitats for biotic colonization and use; therefore, all low-gradient reference were analyzed together. Habitat criteria for low-gradient streams were developed from this entire data set.

The following tentative habitat criteria can be used to determine whether the sampling reach is fully supporting, supporting, but threatened, partially supporting or not supporting its designated use:

1. **Central Appalachian, Southwestern Appalachian and Western Allegheny Ecoregions (High-Gradient Assessments)**

Fully Supporting:	165 and above
Supporting, But Threatened:	164 - 156
Partially Supporting:	155 - 145
Not Supporting:	144 and below

2. **Interior Plateau Ecoregion (High-Gradient Assessments) - Wadeable Streams (>6.0 mi^2)**

Fully Supporting:	145 and above
Supporting, But Threatened:	144 - 131
Partially Supporting:	130 - 105
Not Supporting:	104 and below

3. **Interior Plateau Ecoregion (High-Gradient Assessments) - Headwater Streams (<6.0 mi^2)**

Fully Supporting:	155 and above
Supporting, But Threatened:	154 - 145
Partially Supporting:	144 - 138
Not Supporting:	137 and below

4. **Mississippi Valley Loess Plain and Interior River Hills and Valleys Ecoregions (Low-Gradient Assessments)**

Fully Supporting:	132 and above
Supporting, But Threatened:	131 - 119
Partially Supporting:	118 - 110
Not Supporting:	109 and below

Criteria development is an ongoing process. Updates of the criteria will occur as more reference data is collected.

V. QUALITY ASSURANCE/QUALITY CONTROL

A. Habitat assessment is subjective; however, annual training can help to standardize the assessment.
B. It is best to do the habitat assessment after the biological sampling. This allows the investigator to become more familiar with the types of substrate, degree of embeddedness, the conditions of the banks, etc.
C. If at all possible, have more than one person conduct a habitat assessment. The sampling crew may want to come together and discuss each parameter to attain a final score.
D. If only one person is assessing the habitat, make sure that person continues to conduct the habitat assessments at other sites.
E. Be consistent with your scoring, paying close attention to narrative statements made for each parameter.
F. Repetition will allow you to differentiate between suboptimal and marginal, for instance.
G. Photos of the site could help document habitat conditions and changes.

LITERATURE CITED

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7 - ALGAE

BENTHIC ALGAE

I. INTRODUCTION

Benthic (attached) algae are sensitive indicators of change in lotic waters, as well as being the primary producers within the stream ecosystem. The benthic algae are usually the dominant component of the periphyton. Because it is attached to the substrate, the benthic algae community integrates physical and chemical disturbances to a stream. Another advantage of using benthic algae in water quality assessments is that the benthic algae community contains a naturally high number of species, making data useful for statistical and numerical applications to assess water quality. Response time of benthic algae is rapid, as is recovery, with recolonization after a disturbance often more rapid than for other organisms. Diatoms, in particular, are useful indicators of biological integrity because they are ubiquitous; at least a few can be found under almost any conditions. In addition, most can be identified to species by experienced biologists, and tolerances or sensitivities to specific changes in environmental conditions are known for many species (Dixit, et al. 1992, Rott 1991). By using benthic algal data in association with macroinvertebrate and fish data, the biological integrity of the entire ecosystem can be ascertained.

II. SAMPLING PROCEDURES

A. Benthic Algae Field Data Sheet (Appendix C-1)

Conduct a cursory, visual assessment of the reach and record the results on the benthic algae field data sheet. Record the following station information in the appropriate spaces at the top of the sheet: KDOW station identification number, stream name, location of the station, collection date, time, county, river basin, KDOW program and name of investigator(s). Most of this information can be completed in the office prior to sampling, which will facilitate faster sample collection. After sampling has been conducted, assess the following conditions and record these assessments in the proper spaces: macrohabitats sampled (e.g., riffle, run, pool), microhabitats sampled (Table 7-1), the collection method used, macroalgae present (e.g., *Cladophora*, *Batrachospermum*, *Tetraspora*, etc.), an estimation of benthic algae coverage (e.g., absent, sparse, moderate, heavy), any comments (e.g., whether scour has recently occurred, the flow status of the stream, etc.) and a qualitative ranking of the algal community (See Section I.4.A for details). Use this information to supplement more detailed benthic algal community assessments.

B. Sample Collection

1. Natural Substrates (Headwater and Wadeable streams)

Collect benthic algae from all available microhabitats in wadeable streams (Table 7-1). Collect a composite, qualitative sample from microhabitats in roughly the proportion that they occur at the site. Sample both riffles and pools, or select one major habitat type (usually riffle) if it occurs at all sites to be compared in the study. During low-flow periods, pools may be the only habitat available. Collect samples during stable flow conditions. After extremes of flooding or drought,

allow at least a two-week recolonization period before sampling. Collect samples using the following methods:

a. Use a knife blade, microspatula, toothbrush or similar device to scrape algae from rocks and other hard substrates making every effort to remove all algae from the scraped area. Rinse with distilled water if necessary. Five replicate samples are collected from rocks of similar size whenever possible. Samples from individual rocks can be composited or analyzed separately. Quantitative data can be obtained by measuring the area of substrate sampled.

b. Use a suction device to collect algae from bedrock substrates. Press a section of PVC pipe equipped with a neoprene rubber gasket against the bedrock substrate so that the benthic algae within the enclosed area can be dislodged with a stiff-bristled brush. Suction dislodged material into a filter flask using a hand-operated pump. This method can be used to obtain quantitative data from bedrock (modified from Douglas 1958).

c. Gently lift algal mats from depositional areas with forceps or by suctioning with a disposable pipette.

Table 7-1: Microhabitats Usually Found in Wadeable Streams

Epipelic	Silt and sediment habitats usually in depositional areas with slow current. Algae may form a thin mat that loosely adheres to the surface of the epipelon. To collect epipelic algae, suction material from the mud-water interface using an eye-dropper bulb and a disposable pasteur pipette or gently lift the algal mat from the surface of the sediment using a knife.
Episammic	Sand habitats. Collection is made in the same manner as above.
Epilithic	Rock or other hard surface habitats including dams, bridge abutments, boat ramps, etc. To sample epilithic algae, scrape or hand pick material from epilithon in riffles, pools and runs.
Epidendric	Woody habitats. To collect epidendric algae, scrape or hand pick material from submerged logs, tree roots, drifts, etc.
Epiphytic	Plant habitats usually associated with aquatic mosses, macrophytes and filamentous algae. To sample epiphytic algae, scrape, wring out, hand pick or collect the entire substrate.
Epizooic	Animal habitats including turtle shells, snail shells and other macroinvertebrates. To sample epizooic algae, scrape, hand pick or collect the entire substrate.

2. Artificial Substrate (Non-wadeable Streams)

Algal data from artificial substrates can be used as a tool to assess water quality, even if the natural assemblage is not exactly duplicated (Patrick 1973, Stevenson and Lowe 1986). In non-wadeable streams, rivers with no riffle areas, wetlands or littoral zones of lakes, surface (floating) or benthic (bottom) periphytometers equipped with glass slides are used for collecting algae. Clay tiles, plexiglass plates or other substrates may be substituted for glass slides. Pre-clean glass slides and

rinse with acetone before placing them in the periphytometer. Place artificial substrates in the stream for three to four weeks to allow sufficient time for colonization (Aloi 1990, Weber and Raschke 1970, Weber 1973). To minimize difficulty or errors in analysis of benthic algae from artificial substrates, set a minimum of three periphytometers at each site, from which individual or composite samples can be obtained. Attach periphytometers to trees, snags, bridge pilings or other sturdy anchor sites and away from frequently visited areas (swimming and fishing "holes") to avoid vandalism, theft or other disturbances. Reset periphytometers if flooding or desiccation occur during the exposure period. Allow streams to return to pre-scouring or pre-drought conditions before resetting periphytometers. Samples from replicate periphytometers can be analyzed separately or composited, depending on the goals of the study.

3. Biomass Samples

For biomass samples, scrape natural substrates in the field using distilled water to rinse the substrates. Collect all material and rinse water from the scraped area into an opaque jar and return it, on ice, to the laboratory for filtration of subsamples. Filtration may be performed in the field and filters transported on ice to the laboratory. Do not add preservatives to chlorophyll *a* samples. For natural substrates, measure the area sampled by tracing its outline on paper and determining size with a digital planimeter. Artificial substrates are wrapped in aluminum foil and transported on ice to the laboratory for sample preparation and analysis.

III. SAMPLE PRESERVATION AND LABELING

Collect samples in small, watertight glass vials or screw-top bottles of at least 60 ml in volume. Preserve taxonomic samples with 3% to 4% buffered formalin, 2% glutaraldehyde, Lugol's solution or other preservatives listed in the latest edition of Standard Methods (APHA 1992). Chlorophyll *a* and ash-free dry-weight samples should be placed on ice only. Affix a label permanently to the sample container, recording the following information on the label using a pencil or waterproof, indelible ink: waterbody name, location, sample number, date and name of collector. Record this information in a laboratory sample logbook (Appendix C-2). Refrigerate samples until taxonomic evaluation is completed. After taxonomy is completed, archive the samples in a cool, dry place. Permanent mounts of diatom samples are retained in wooden or plastic slide boxes.

IV. SAMPLE ANALYSIS

A. Field Assessment

While in the field, qualitatively rank the benthic algal community at each station. A score of 1 (lowest quality) to 5 (highest quality) is possible. Record scores and a description of the benthic algal community on the benthic algae field data sheet (Appendix C-1). While somewhat subjective, this information can be used later to support a detailed assessment of the benthic algal community. The algae are carefully judged using the following ranking criteria:

1) **Excellent Quality (5)**: The benthic algal community appears diverse with several divisions represented, including chrysophytes, chlorophytes, cyanophytes and rhodophytes. Phytoplankton sub-community are not apparent. Floating algal mats are not present. The algal community is similar to that of reference stations within the same ecoregion.

2) **Fair - Good Quality (2-4)**: Benthic algae are present in moderate amounts. The benthic algal community may be dominated by one type of growth, such as long filaments of *Cladophora*. Diversity is low to moderate, and a phytoplankton sub-community is not apparent. Floating algal mats may be present, but are not extensive. Clean water benthic algal taxa (e.g., red algae, *Chaetophora*, etc.) present in reference reach stations may not be present.

3) **Poor Quality (1)**: In cases of toxic pollution (acid mine drainage, toxic discharges, etc.), substrates and water column may appear sterile, bleached or rust-colored. Little or no algae are observed. With organic pollution (sewage discharges, etc.), substrates may be covered with thick white, black or gray mats of filamentous bacteria, thick algal mats of cyanophytes (blue-green algae) and/or chlorophytes (green algae). The water column may have a "pea green" appearance as a result of high abundances of euglenophytes, or large floating mats of algae may be present, especially in pools and slow-moving streams. Look for extremes of either characteristic. Diversity is very low. Very few, if any, clean water taxa are present.

B. Laboratory Analysis

1. Non-Diatom Algae Slide Preparation and Analysis

Equipment:
Microscope with 20X and 40X objectives
Glass microscope slides
Glass coverslips
Watchglass
Disposable pipettes

Procedure:
In this document, the term "non-diatom algae" refers to all taxa that do not belong to the Class Bacillariophyceae. Thoroughly shake the sample container to dislodge epiphytes from filamentous taxa and randomly mix all algal organisms. Pour the contents into a shallow watchglass so that all filamentous and mat-forming taxa can be separated. Using dissecting probes or needle-nosed forceps, place representative filamentous taxa on a pre-cleaned microscope slide or in a settling chamber for inverted microscope use. Cover the filaments with approximately 0.5 ml of sample liquid. Gently place a coverslip over the subsample, completing the wet mount. First, examine each slide at 200x, then at 400x to ensure that smaller organisms are not overlooked. Identify all non-diatom algae to the lowest taxonomic level using current taxonomic references. Scan each slide until no new organisms are seen. Examine a minimum of three slides for each sample. Record observed taxa on the non-diatom bench sheet (Appendix C-3) along with taxonomic division, estimated relative abundance (abundant, common, rare) and any known autecological information. In some instances, counts of the non-diatom community may be needed in order to obtain specific autecological information. Homogenize the sample with a blender. Pipette a subsample into a Palmer counting cell. Identify and count 300 algal non-diatom units to the lowest taxonomic level at 400X. Non-diatom algal units are considered instead of cells. Since colonial, coenobial or filamentous cells normally do not occur singly in a natural environment, counting each cell does not accurately portray relative abundance for that specific taxon. For example, although *Pediastrum*

duplex is composed of several cells, the colony as a whole is counted as one unit. Likewise, coenobia and unicells are each considered one unit. Filaments may be counted either as one unit or counted in units of 10 um lengths (A 100-um filament is 10 units.) Record numbers of non-diatom algal units on the non-diatom bench sheet. Enter the data into an appropriate database, such as EDAS (Ecological Data Application System). Taxonomic references are listed at the end of this chapter.

2. Diatom Slide Preparation and Analysis

A. Cleaning Methods for Diatoms

After the non-diatom algae have been identified, clear diatom frustules of organic and intercellular material using one of the following oxidation methods (APHA 1992, van der Werff 1955):

1. Nitric Acid Oxidation

Equipment:
2000 ml Erlenmeyer flask
1000 ml graduated cylinder
20-30 ml of algae sample
50 ml of HNO_3

Procedure:
Shake the sample vigorously and immediately pour a portion (about 20-30 ml) of the sample into a large 2000 ml Erlenmeyer flask. Under a flame hood, add 50 ml of concentrated HNO_3. Allow the sample to oxidize overnight, then fill the flask with distilled water. Let the sample resettle overnight, siphon off the supernatant, and pour the remaining diatom solution into a 1000 ml graduated cylinder. Fill with distilled water, settle and siphon supernatant at least twice more, until the yellow color changes to clear.

2. Hydrogen Peroxide/Potassium Dichromate Oxidation

Equipment:
2000 ml Erlenmeyer flask
1000 ml graduated cylinder
20-30 ml algae sample
50 ml of 50% H_2O_2
Microspatula
Dash of $K_2Cr_2O_7$

Procedure:
Prepare sample as in nitric acid method, but use 50 ml of 50% H_2O_2 instead of HNO_3. Allow to oxidize overnight; then add a microspatula of $K_2Cr_2O_7$. This will cause a violent exothermic reaction, so be careful to perform this method only under a fume hood. When

the sample color changes from purple to yellow and boiling stops, fill the flask with distilled water. Repeat rinsing steps as outlined for the HNO_3 method until the yellow color is gone.

B. Diatom Slide Preparation

Equipment:
Pre-cleaned microscope slide
Glass coverslip
Naphrax mounting medium
Hot plate

Procedure:
Using a disposable pipette, drop a small amount of well-mixed sample onto a coverslip that has been placed on a hot plate. Dry at low heat until the moisture is removed. Place a large drop of Naphrax mounting medium onto a pre-cleaned microscope slide. Invert the coverslip onto the Naphrax. Place the slide on a hot plate on high heat. Allow the toluene to boil out of the Naphrax. Make sure that this procedure is conducted in or near a laboratory hood where ventilation is good. After bubbles of toluene have stopped forming, remove the slide from the hot plate. Allow the slide to cool and harden. Remove all excess Naphrax from the slide with a razor blade. Affix a slide label to the slide and place in a slide box for storage.

C. Diatom Identification and Enumeration

Equipment:
Microscope with 100X oil immersion objective
Immersion oil

Procedure:
Identify diatoms at 1000X to the lowest possible taxonomic level, preferably to the species or variety level, using current taxonomic references. Record all taxa encountered on the diatom bench sheet (Appendix C-4) creating a species list prior to enumeration. Scan the slide until several minutes pass without producing any new taxa. For quantitative data, count a minimum of 500 valves recording taxa and number counted on the diatom bench sheet. Data assessment is based on the completed species list. Enter data into an appropriate database, such as EDAS.

D. Biomass

A. Chlorophyll *a*

Chlorophyll *a* analyses are performed as described for phytoplankton (Chapter 3, Part II), using U.S. EPA Method 445.0 (USEPA 1992).

B. Ash-Free Dry-Weight (AFDW)

Replicate samples are analyzed in accordance with Standard Methods (APHA 1992).

V. DATA ANALYSIS

An assessment of biological integrity can be made based on the benthic algal data. The goal is to categorize water quality as excellent, good, fair or poor and to determine the degree and cause of aquatic life use impairments in fair or poor streams. A multiple metric index called the Diatom Bioassessment Index (DBI) is used to assess the benthic algal community.

A. Diatom Bioassessment Index

Biological indices represent mathematical models of community changes (Perkins 1983). Changes in water quality will affect resident biota, and indices that reflect these changes in a particular community are useful biological indicators of water quality. The benthic algal community, especially diatoms, is a useful biological indicator because: 1) they are attached to the substrate and, therefore, subjected to any immediate or prolonged disturbances; 2) diatoms are ubiquitous, with at least a few individuals found under almost any aquatic conditions; 3) total number of taxa at any given site is usually high enough for use in calculating various metrics; 4) diatoms, especially the most abundant species, are identifiable to species by trained professionals; 5) tolerance of or sensitivity to changes (autecological requirements) is known or suspected for many species or assemblages of diatoms; and 6) bethic algal communities, especially diatoms, have a rapid response and recovery time because of their relatively short lifecycle (as compared to fish or macroinvertebrates) and their ability to quickly recolonize formerly disturbed (impacted) sites (Dixit et al. 1992).

Several metrics have been used to assess water quality conditions using benthic algae. Some have the diagnostic ability to indicate the type of impact (nutrient enrichment, toxicity, acidity, salinity, sewage (organic) pollution and siltation). However, each metric alone could not accurately describe the overall water quality at a site. In the mid-1980s, phycologists at the KDOW developed a multi-metric index using four benthic diatom metrics. This multi-metric approach was modeled after successfully used indices such as Karr (1981) with fish and Plafkin, et al. (1989) with macroinvertebrates. The metrics chosen for the original Diatom Bioassessment Index (DBI) were as follows: total number of diatom taxa (TNDT), Shannon diversity (H′), pollution tolerance index (PTI) and % sensitive species (%SS). Recently, phycologists at KDOW have refined the DBI using box and whisker plots to test metric sensitivity and the Pearson's correlation coefficient index to test for metric redundancy. A new DBI was developed using three of the original metrics (TNDT, H′, and PTI) and the following three new metrics: *Cymbella* group richness (CGR), *Fragilaria* group richness (FGR), and % *Navicula*, *Nitzschia* and *Surirella* (%NNS). The new DBI provides water resource managers with a very sensitive, community structure-based tool for assessing water quality.

1. Diatom Bioassessment Index Metrics

a. Total Number of Diatom Taxa (TNDT)

Total number of diatom taxa (TNDT) is an estimate of diatom species richness. High species richness is assumed to be the case in an unimpacted site, and species richness is expected to decrease with increasing pollution. Slight levels of nutrient enrichment, however, may increase species richness in naturally unproductive, nutrient-poor streams

(Bahls 1992). Low-order, pristine streams in the Central Appalachian or Western Allegheny ecoregion of eastern Kentucky may fall into this category.

i. TNDT = total number of diatom taxa identified

ii. TNDT/500 = total number of diatom taxa encountered in a count of 500 individuals.

b. Shannon Diversity

The mean Shannon diversity index is used in diatom assessments. It was chosen primarily because it is commonly used by many aquatic biologists, so values will be more readily interpreted and compared with other literature values. Using this index, H′= 0 when only one species is present in the collection, and H′ is at a maximum when all individuals are evenly distributed among the S species.

$$H' = - \sum \frac{n_i}{N} \log_{10} \frac{n_i}{N}$$

where:
n_i = number of individuals of species i
N = total number of individuals

H′ can also be expressed using natural logarithms (ln); however, $\log_{10}$ is used for historical comparison with other data collected across the state (Harker et al. 1979). Conversion factors for ln and $\log_2$ are available in Weber (1973).

i. Disadvantages

Diversity is affected by both the number of species in a sample and the distribution of individuals among those species (Klemm et al. 1992). Because species richness and evenness may vary independently, under certain conditions diversity values can be misleading. For example, streams with poor water quality due to toxic discharges may have very low taxa richness, but the individuals present may be very evenly distributed among those few taxa. This often results in high diversity values under stressed water quality conditions (KDOW unpublished data, Pontasch and Brusven 1988, Pontasch et al. 1989). Archibald (1972) also warns of the dangers of using diatom diversity alone as an indicator of water quality because low diversity values may indicate either heavily polluted water or clean water. Hurlbert (1971) goes so far as to state that species diversity is an ecologically meaningless concept and suggests its use be abandoned; and according to Perkins (1983), the assumption that individuals in more polluted environments should be less evenly distributed is speculative. It can be argued that the evenness assumption of the diversity index may be ecologically unsound and that in natural communities individuals are not evenly distributed among species. This is supported by the work of Patrick et al. (1954) on the structure of natural diatom communities.

ii. Advantages

Species diversity, despite the controversy surrounding it, has historically been used with success as an indicator of organic (sewage) pollution (Wilhm and Dorris 1966, Weber 1973, Cooper and Wilhm 1975). Bahls (1992) uses Shannon diversity because of its sensitivity to water quality changes, and Stevenson (1984) suggests that changes in species diversity, rather than the diversity value, may be useful indicators of changes in water quality. It is obvious that successful use of the diversity index depends upon careful application and interpretation. As a metric used in the DBI, it is only one of several metrics and will not be used alone as a water quality indicator.

c. Pollution Tolerance Index (PTI)

Several recent water quality indices based on pollution tolerance (or sensitivity) of diatom species have been proposed, including the saprobity index of Sladecek (1973), and diatom pollution tolerance indices developed by Lange-Bertalot (1979), Descy (1979), Leclercq and Maquet (1987) and Watanabe et al. (1988). What these indices have in common is that species are differentiated into groups and assigned values relating to pollution tolerance. Calculations involve estimates of the dominance of species and a "subjective assessment of the value of each species as an indicator" (Round 1991). Other state agencies in the U.S. using some type of diatom pollution tolerance index include the Montana Water Quality Bureau (Bahls 1992) and Oklahoma Conservation Commission (Bob Lynch, pers. comm.). Ideally, a consensus will be reached on which diatom pollution tolerance index should be used, and a common list of pollution tolerance values will be developed. However, because a species may react differently across different ecoregions and may have variable sensitivities to different types of pollution (e.g., nutrients, metals, pH, salinity), universal values may not be appropriate.

The pollution tolerance index (PTI) used by the Kentucky Division of Water is most similar to that of Lange-Bertalot (1979) and resembles the Hilsenhoff Biotic Index for macroinvertebrates (Hilsenhoff, 1987). Lange-Bertalot distinguished three categories of diatoms according to their tolerance to increased pollution, with species assigned a value of 1 for most tolerant taxa (e.g., *Nitzschia palea* or *Gomphonema parvulum*) to 3 for relatively sensitive species. For the PTI, Lange-Bertalot's list has been adapted to four categories to differentiate a large moderately tolerant group of species (similar to his splitting of category 2 diatoms into 2a and 2b); the KDOW diatom pollution tolerance values range from one (most tolerant) to four (most sensitive). Tolerance values for Kentucky diatoms were generated from a multitude of literature, including Lowe (1974), Patrick and Reimer (1966 and 1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater, et al. (1988), Bahls (1992), Mississippi Department of Environmental Quality (Stanley Rogers, pers. comm.) and Oklahoma Conservation Commission (Bob Lynch, pers. comm.). The extensive KDOW diatom database collected from 1977 to the present and data collections by the Kentucky Nature Preserves Commission (1979 - 1986) were also instrumental in the determination of tolerance values. The list of tolerance values presently in use (Appendix C-5) will be revised and updated as new autecological data is discovered; however, the tolerances of most common species are fairly well understood. Because the index is based on relative abundances, rare species will have little effect on the final index value. If no autecological data is known, the species is given a PTI value of 0 and is not used in PTI index calculation.

The formula used to calculate PTI is:

$$PTI = \frac{\sum n_i \; x \; t_i}{N}$$

where n_i = number of individuals in species i
t_i = tolerance value of species i
N = total number of individuals

d. Siltation Index (%NNS)

The sum of the relative abundances of all *Navicula* (including *Aneumastus*, *Cavinula*, *Chamaepinnularia*, *Cosmioneis*, *Craticula*, *Diadesmis*, *Fallacia*, *Fistulifera*, *Geissleria*, *Hippodonta*, *Kobarasia*, *Luticola*, *Lyrella*, *Mayamaia*, *Muellaria*, *Placoneis* and *Sellaphora*), *Nitzschia* (including *Psammodictyon* and *Tryblionella)* and *Surirella* taxa reflects the degree of sedimentation at a reach. These three genera are motile, using their raphes to slide through sediment if they become covered. Their abundance expresses the frequency and severity of sedimentation. As sedimentation increases, the %NNS is expected to increase (Bahls et al. 1992).

%NNS = the sum of the relative abundances of all *Navicula*+*Nitzschia*+*Surirella* taxa

e. Fragilaria Group Richness (FGR)

The total number taxa represented in the sample from the genera *Ctenophora*, *Fragilaria*, *Fragilariforma*, *Pseudostaurosira*, *Punctastriata*, *Stauroforma*, *Staurosira*, *Staurosirella*, *Tabularia* and *Synedra* reflects high water quality. As water pollution increases, the FGR is expected to decrease.

FGR=*Ctenophora*+*Fragilaria*+*Fragilariforma*+*Pseudostaursira*+*Punctastriata*+*Stauroforma*+ *Staurosira*+*Staurosirella*+*Synedra*+*Tabularia*

f. Cymbella Group Richness (CGR)

The total number of taxa represented in the sample from the genera *Cymbella*, *Cymbopleura*, *Encyonema*, *Encyonemopsis*, *Navicella*, *Pseudoencyonema* and *Reimeria* reflects high water quality. As water pollution increases, the CGR is expected to decrease.

CGR=*Cymbella*+*Cymbopleura*+*Encyonema*+*Encyonemopsis*+*Navicella*+*Pseudoencyonema*+ *Reimeria*

2. Future Diatom Bioassessment Index Metrics

Other metrics may be included in the new DBI as their usefulness is evaluated and more data are collected.

a. Total Number of All Algal Genera (TNG)

Total number of all algal genera (TNG) may provide a better estimate of diversity than taxa richness. As water pollution increases, TNG is expected to decrease (Barbour et al. 1999).

TNG = total number of benthic algal genera identified (non-diatom + diatom)

b. Total Number of Divisions Represented (TDiv)

Representatives from several divisions of algae are common from sites with good water quality. The number of divisions represented is reported as an indicator of diversity.

3. Calculating the Diatom Bioassessment Index

Each metric is given a calculated score (range 0-100) based on the percent of the standard metric value (i.e., the 95th percentile or 5th percentile) of the entire database (impaired and reference). These percentile thresholds are used to eliminate outliers. The formulae for calculating DBI scores are shown in Table 7-2.

Table 7-2: Metric Scoring Formulae for the Diatom Bioassessment Index

Metric	Formula
TNDT	(TNDT/95th%ile) X 100
H′	(H′/95th%ile) X 100
PTI	(PTI/95th%ile) X 100
FGR	(FGR/95th%ile) X 100
CGR	(CGR/95th%ile) X 100
%NNS	(100 - %NNS)/(100 - 5th%ile) X 100

Metric scores with values greater than 100 receive a score of 100.0. The mean of the six DBI metrics is the final DBI score on a 0-100 scale.

Final DBI scoring criteria are currently being developed for each ecoregion in Kentucky using an extensive KDOW database collected from 1986 through 2000. Refinement of these criteria will continue as more data are collected.

B. Other Data Evaluation Methods

Data assessment and interpretation is by no means restricted to the above metrics. Statistical analyses, when appropriate, may render useful insights into benthic algal data. Often, however, biological data are not appropriate for parametric statistical analyses because the basic assumption of normal distribution is not always met. In addition, statistically significant differences are not always ecologically significant (APHA 1992, Elliott 1983). Multivariate analyses are becoming popular in environmental assessments (Ter Braak 1988, Gauch 1982) and as user-friendly software packages become available, these methods will certainly be explored in the future. Such methods are especially useful in determining the tolerances of benthic algal species to specific environmental parameters (Agbeti 1991, del Giorgio et al. 1991, Dixit et al. 1992 and Sabater et al. 1988).

1. Chlorophyll *a*

Benthic chlorophyll *a* values are used as an estimate of algal biomass. Chlorophyll *a* values can be extremely variable because of the patchiness of benthic algal distribution; therefore, assessments are

based on a mean of three or more replicate samples. These values are used to compare biomass accrual at the same station over time or between stations during the same sampling period. High chlorophyll *a* values may indicate nutrient enrichment, while low values may either indicate low nutrient availability, toxicity or low-light availability because of shading, sedimentation or high turbidity. Chlorophyll *a* values are used only in support of other analyses.

2. **Ash-free Dry-weight (AFDW)**
   Benthic AFDW values are used as an estimate of total organic material accumulated on the artificial substrate. This organic material includes all living organisms (algae, bacteria, fungi, protozoa and macroinvertebrates) as well as non-living detritus. Ash-free dry-weight values have been used in conjunction with chlorophyll *a* as a means of determining the trophic status (autotrophic vs. heterotrophic) of streams. The Autotrophic Index (AI) is calculated as follows:

$$AI = \frac{AFDW\ (mg/m^2)}{Chlorophyll\ a\ (mg/m^2)}$$

   High AI values (>200) indicate the community is dominated by heterotrophic organisms, and extremely high values indicate poor water quality (Weber 1973, Weitzel 1979, Matthews et al. 1980). This index should be used with discretion, as non-living organic detritus can artificially inflate the AFDW value.

VI. QUALITY ASSURANCE AND QUALITY CONTROL

A. Quality Assurance and Quality Control in the field (Barbour et al. 1999)

1. Make sure that sample labels are accurate and complete with all pertinent information that were previously discussed.

2. Before leaving a reach, all sampling equipment will be checked for residual algal material, rubbed clean and thoroughly rinsed with distilled water.

3. One duplicate sample from 10% of the reaches sampled within a sampling season will be collected and analyzed to ensure precision and repeatability of the sampling technique.

4. Phycologists are trained in the sampling techniques on a regular basis.

B. Quality Assurance and Quality Control in the laboratory (Barbour et al. 1999)

1. Record all samples in the laboratory sample logbook (Appendix C-2).

1. A voucher collection will be maintained of all samples and diatom slides. Label information must be accurate and complete.

2. Phycologists should discuss any problem identifications with other phycologists. Any problem taxa should be sent to an outside taxonomist to assist in identification.

3. Duplicate samples will be processed and analyzed in the same procedures as regular samples.

4. Another in-house phycologist should identify and enumerate the duplicate samples. If there is not another phycologist in the lab, an outside phycologist should be utilized to periodically check identification of samples.

5. Duplicate samples should exceed 75% similarity (Whittaker 1952) of the replicate sample.

6. The most recent taxonomic references will be used for the identification of algae.

7. Phycologists will participate in algal identification workshops, seminars and training sessions when opportunities arise.

PHYTOPLANKTON

I. INTRODUCTION

Phytoplankton are important primary producers in lakes, impoundments, ponds, wetlands, low-gradient streams and backwater areas. *Lotic* phytoplankton samples may be collected by the KDOW from biological monitoring stations and from selected intensive survey sites. In addition, phytoplankton samples are collected from streams and reservoirs used as drinking water supplies if taste and odor problems occur. Samples are analyzed for chlorophyll *a*, phytoplankton density (cells/ml) and/or phytoplankton community structure, depending upon the nature of the survey. *Lentic* phytoplankton samples may also be collected to assess water quality and recreation uses.

II. SAMPLING PROCEDURES

A. Phytoplankton Field Data Sheet (Appendix D-6)

Conduct a cursory, visual assessment of the reach and record the results on the phytoplankton field data sheet. Record the following station information in the appropriate spaces at the top of the sheet: KDOW station identification number, waterbody name, location of the station, collection date, time, county, river basin, purpose of the study (e.g., chlorophyll *a*, taste and odor, etc.) and name of investigator(s). Most of this information can be completed in the office prior to sampling, which will facilitate faster sample collection. After sampling has been conducted, record the collection method used, analyses to be conducted (e.g., chlorophyll *a*, ash-free dry-mass, phytoplankton count), bloom characteristics (e.g., color of surface scum, any odor present, any surface sheen present, thickness of the bloom, etc.) and the surface bloom coverage (See Section II.4.A) in the appropriate spaces on the phytoplankton field data sheet.

B. Sample Collection

Collect samples in one-liter glass or plastic containers. Samples from wadeable streams are collected near mid-stream, approximately 0.5 m beneath the surface. Composite, euphotic zone samples are collected from unwadeable streams, wetlands, lakes and impoundments using a Kemmerer or Van Dorn sampler. Skim surface scums formed by algal blooms for additional algal identification when present.

C. Chlorophyll *a* Samples

For chlorophyll *a* analysis, filter 50 to 100 ml aliquots of the phytoplankton sample through 2.4 cm Whatman GF/C glass microfibre filters. Fold filters in half, with algal cells folded to the inside. If filtering is performed in the field, place filters in light-excluding containers, such as empty 35mm film containers, or wrap filters in aluminum foil and place them in a zip-lock bag. Preserve samples on ice in a cooler until they can be returned to the laboratory. As an alternative to filtering in the field, whole-water samples (1 L or greater) may be preserved on ice and filtered upon return to the laboratory. DO NOT add chemical preservatives to samples to be analyzed for chlorophyll *a*. Samples should be filtered within 24 hours of collection and immediately frozen.

III. SAMPLE PRESERVATION

Samples for identification and enumeration are preserved with 3 to 4% buffered formalin, Lugols solution, 2% glutaraldehyde, M^3 fixative or 6-3-1 preservative. Samples should be labeled with waterbody name, location, site number, date and name of collector. Labels should be written in pencil or indelible ink and attached firmly to the container.

IV. SAMPLE ANALYSIS

A. Field Assessment

A *qualitative* field determination of surface algal bloom coverage is recorded on the field data sheet using the following criteria:

Total - 100% (bank to bank)
Dense - 60% to 99%
Moderate - 30% to 59%
Sparse – 1% to 29%
Absent - 0%

B. Primary Productivity

If primary productivity studies take place, the procedures followed will be those described in the latest edition of Standard Methods (APHA 1992).

C. Chlorophyll *a* Analysis

1. Filtration, Extraction and Determination

Replicate samples will be analyzed using a Turner Designs Model 10 fluorometer, using U.S. EPA Method 445.0 (U.S. EPA 1992). The fluorometric method is more sensitive than the spectrophotometric method, and smaller sample volumes can be analyzed.

a. **Filtration**

Equipment:
Hand-operated or electric pump at a vacuum pressure of less than 6 in of mercury.

500 ml filter flask
50 ml filter funnel
2.4 cm Whatman GF/C glass microfibre filters

Procedure:

Phytoplankton - filter 50 to 100 ml of sample through a 2.4 cm Whatman GF/C filter.

Periphyton - scrape a glass microscope slide from periphytometer into a watch glass, rinsing the slide with distilled water. Filter the resulting solution, rinsing the watch glass with distilled water. Remove the filter from the funnel, fold it in half, and place it in a light-excluding container (e.g., 35 mm film container). Repeat the process for each replicate sample. For natural substrate samples, scrape a known area of substrate into a jar and record the volume of sample. Filter a subsample of 1 to10 ml, depending upon the amount of algae present in the sample. Mechanical blending may be necessary to homogenize the sample if large mats or filaments are present. For storage, freeze filters at 4^{o}C for no longer than 30 days.

b. Extraction

Reagents:
90% aqueous acetone (mix 900 ml reagent grade acetone with 100 ml distilled water)

Equipment:
Tissue grinder with teflon pestle
Glass-grinding vessel with a round bottom that matches the teflon pestle
Plastic centrifuge tubes, graduated

Procedure:
Place one filter in the grinding vessel and add 5 ml of acetone solution and macerate the filter. Pour the resulting solution into a centrifuge tube. Rinse the grinder with another 5 ml of the acetone solution and transfer to the centrifuge tube. Steep samples in a dark refrigerator for at least 2 hours, but no more than 24 hours. Centrifuge at 1000 g for 5 to 20 minutes to clarify the extract.

c. Analysis

Reagents:
90% aqueous acetone
2 N HCl (for acid-corrected method)

Equipment:
Turner Designs model 10 Fluorometer equipped with chlorophyll kit filters and bulb (as recommended by the manufacturer)
Glass cuvette

Procedure:
Calibrate the fluorometer at each sensitivity level annually, or whenever filters or lamps are changed, using directions and samples obtained from the U.S. EPA Quality Assurance Lab in Cincinnati, Ohio. If necessary, prepare 0.1 or 0.01 dilutions of the sample if the chlorophyll *a* extract sample is too concentrated. Usually, phytoplankton samples from streams need no dilution. Phytoplankton from enriched streams or eutrophic lakes may need dilutions of 0.1, and periphyton samples may need to be diluted by a factor of 0.01.

d. Acidification method for corrected chlorophyll *a*:
Fill cuvette about 2/3 full with chlorophyll extract. Record the initial reading as Rb (reading before acidification). Record the instrument sensitivity level and keep readings between 20 and 80 by either changing the sensitivity or diluting the sample. Remove the cuvette and add 3 drops of 2N HCl. Gently agitate, allowing approximately 60 seconds for mixing and degradation of chlorophyll *a* to phaeophytin, then record, from the same scale as before, Ra (reading after acidification).

The formula used for calculating corrected chlorophyll *a* is:

$$\text{Chl } a = F_s \frac{r}{r - 1} (Rb - Ra)$$

where:
F_s = calibration factor for each sensitivity level
Rb = reading before acidification
Ra = reading after acidification.
$r = R_b/R_a$ (when the instrument is calibrated using pure chlorophyll standards of known concentration)

Record the results on a chlorophyll *a* bench sheet (Appendix D-7).

e. Uncorrected chlorophyll *a*
To calculate chlorophyll *a* using narrow band filters, use the formula:

$$\text{Chl } a = \frac{F_s \, r \, Rb}{r-1}$$

Acidification of the sample is not necessary if this formula is used.
Record the results on a chlorophyll *a* bench sheet (Appendix D-7).

D. Identification and Enumeration

Phytoplankton is concentrated by settling, identified to the lowest possible taxon using appropriate taxonomic references, and if *quantitative* data are required, enumerated as follows:

1. Place an aliquot of concentrated sample into a calibrated counting chamber such as a Sedgewick-Rafter cell or a sedimentation cylinder with a standardized bottom area (inverted microscope technique).

2. Allow the sample to settle for an appropriate amount of time. For 1 ml of sample, allow a sedimentation time of one hour; for greater volumes of sample, increase the sedimentation time to ensure settling of all phytoplankton.

3. Identify and enumerate phytoplankton with a binocular compound microscope or an inverted phase-contrast microscope equipped with a standard Whipple grid. Examine sedimentation chambers at 400X on an inverted microscope. Examine Sedgwick-Rafter counting chambers at 200X on a compound microscope. A list of taxonomic references follows the text.

4. Count a minimum of 200 algal units for each sample. Report results for each taxon as a relative percentage of the total count (relative abundance). Algal density, biovolume, taxa richness, diversity, percent similarity or other metrics will be calculated when needed.

V. INTERPRETATION OF PHYTOPLANKTON DATA

A. Chlorophyll *a*

Chlorophyll *a* data are compared with other sites in the same drainage, reference reach sites, or intensive survey control sites. Chlorophyll *a* data will also be used for biological trend monitoring.

B. Community Structure

Phytoplankton abundance will be used to determine the degree and extent of algal blooms. Metrics such as taxa richness, relative abundance, community similarity, diversity, or other appropriate measures of phytoplankton community health will be evaluated and compared to other sites in the drainage, reference or control sites, available historical data and appropriate scientific literature.

C. Use Impairments

Algal blooms will be evaluated to determine use impairment for Aquatic Life, Recreational and Domestic Water Supply Uses, as well as the minimum criteria applicable to all surface waters. These uses are defined in Kentucky's surface water quality standards (401 KAR 5:031).

1. **Aquatic Life** - the autecology of dominant and abundant taxa and associations will be determined when information is available (Anonymous 1967, Bennet 1969, Collins and Weber 1978, Lowe 1974, Palmer 1977, Patrick 1977, Patrick et al. 1975, VanLandingham 1982, Whitford and Schumacher 1963). Presence of the following indicator groups will be recorded:

heterotrophic taxa - indicative of organically enriched conditions.
halophilic taxa - indicative of high salinities
acidophilic taxa - indicative of low pH

2. **Domestic Water Supply** - taste and odor algae are identified and enumerated when appropriate. Potentially toxic species will be identified and enumerated.

3. **Recreational Use** - algae will be identified and enumerated. Algal blooms and bloom potential will be evaluated using chlorophyll *a*, cell density or both types of data.

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8 - MACROINVERTEBRATES

I. INTRODUCTION

A. Macroinvertebrates as Environmental Indicators

Since the early 1900s, aquatic organisms have been used extensively in water quality monitoring and impact assessment (Cairns and Pratt 1993), and macroinvertebrate assemblages have proven to be very useful in detecting even the subtlest changes in habitat and water quality. Today, macroinvertebrates are used throughout the world in water quality assessment, as environmental indicators of biological integrity, to describe water quality conditions or health of the aquatic ecosystem, and to identify causes of impairment. The KDOW relies on the analyses of macroinvertebrate communities in use-attainment designations for sections 305b and 303d of the Clean Water Act (CWA) reporting, assessing specific effects of pollutants on water quality and for listing Exceptional Water designations to streams and rivers throughout the Commonwealth. KDOW defines benthic macroinvertebrates as organisms large enough to be seen by the unaided eye, can be retained by a U.S. Standard No. 30 sieve (28 mesh/inch, 600 µm openings) and live at least part of their life cycle within or upon available substrates of a waterbody.

B. Type of Collections

The WQB typically collects macroinvertebrates either to assess baseline water quality or to make a bioassessment of areas impacted by anthropogenic activities. In addition, a random survey approach is used to assess aquatic life use-support for streams in each watershed management unit (see Section 2.E.). Standardized, semi-quantitative collections are made at all sampling locations; however, precise quantitative data may be collected on a case-by-case basis or if biological monitoring requires more rigorous statistical analyses. In general, collection methods used by KDOW are similar to those discussed in Lenat (1988), Plafkin et al. (1989), Klemm et al. (1990), Eaton and Lenat (1991), USEPA (1997b) and Barbour et al. (1999).

C. When to Sample

Adherence to sample index periods is very important for accurate bioassessments using macroinvertebrates since biocriteria are calibrated for seasonality. For ***wadeable*** and ***non-wadeable*** streams, sampling must occur from May through September. For ***headwater*** streams (<5 mi^2 in drainage area), sampling must take place from February through May. In some cases, sampling outside of these index periods is necessary to assess immediate impacts (e.g., chemical spills) or to adhere to specific guidelines set forth by the U.S. Fish and Wildlife Service and KDOW Standards and Specifications Section for trend monitoring and bioassessments in streams containing Federally Listed species. For routine bioassessments or baseline data collection, samples collected outside of these index periods may be considered unacceptable. Also, biological samples should not be collected during periods of excessively high or low flows or within two weeks of a scouring flow event.

II. SAMPLING METHODS

The methods described in this section are modifications of the single and multi-habitat approaches outlined by Barbour et al. (1999). The methods vary somewhat among stream sizes and stream gradients because of differences in habitat types present. The WQB of KDOW must approve any deviation from the methods described below. For multiple-site intensive surveys of known impacts, and for comparison to regional reference conditions, the 1-m^2 kicknet samples should be kept separate from other habitat collections to facilitate data interpretation, provide for better diagnosis of impairment and minimize the influence of uncontrolled variables.

A. Wadeable-Moderate/High Gradient

For macroinvertebrates, KDOW generally considers wadeable streams as 3rd order or larger and usually greater than 5 square miles (13 km^2) in drainage area. A summary of collection techniques required for these types of streams is shown in Table 8-1. Wadeable low-gradient streams, high-gradient headwater streams and large non-wadeable rivers require different methods and are discussed in Sections B, C and D, respectively. For routine and intensive surveys, it is important that both riffle/run samples and multi-habitat sampling are conducted (see below). As a quick screening tool, a single habitat (riffle/run) sample may be taken to detect obvious impairment from reference conditions. This simplified method may be employed for identifying impacted areas for TMDL devlopment.

1. Riffle Sample

This technique samples the most important subhabitat (i.e., riffle) found in moderate to high-gradient streams. It requires using a 600 µm mesh, one meter wide net in moderate to fast current in areas with gravel to cobble substrate. Four (4) 0.25 m^2 samples are taken from mid-riffle or the thalweg (path of deepest thread of water), dislodging benthos by vigorously disturbing 0.25 m^2 (20 x 20 in.) in front of the net. Large rocks should be hand washed into the net. The contents of the net are then washed and all four samples are composited into a 600 µm mesh wash bucket. ***This sample must be kept separate from all other subhabitat collections.***

2. Mutli-Habitat Sample

a) Sweep Sample - This sample involves sampling a variety of non-riffle habitats with the aid of an 800 x 900 µm mesh triangular or D-frame dipnet. Each habitat is sampled in at least three (3) replicates, where possible.

1) Undercut banks/root mats - sampled by placing a large rootwad into the triangular or D-frame dipnet and shaken vigorously. The contents are removed from the dipnet and placed into a mesh wash bucket. Note: if undercut banks are present in both run and pool areas, each is sampled separately with three replicates.

2) Marginal emergent vegetation (exclusive of *Justicia americana* beds) - sampled by thrusting (i.e., "jabbing") the dipnet into the vegetation for ca. 1 m, and then sweeping through the area to collect dislodged organisms. Material is then rinsed in the wash bucket and any sticks, leaves and vegetation are thoroughly washed and inspected before discarding.

3) *Bedrock or slab-rock habitats* - sampled by placing the edge of the dipnet flush on the substrate, disturbing approximately 0.1 m^2 of area to dislodge attached organisms. Material is emptied into a wash bucket.
4) *Justicia americana (water willow) beds* - sampled by working the net through a 1 m section in a jabbing motion. The material is then emptied into a wash-bucket and any *J. americana* stems are thoroughly washed, inspected and discarded.
5) *Leaf Packs* - preferably "conditioned" (i.e., not new-fall material) where possible; samples are taken from a variety of locations (i.e., riffles, runs and pools) and placed into the wash-bucket. The material is thoroughly rinsed to dislodge organisms and then inspected and discarded.

b) Silt, sand, and fine gravel

1) *Sieving* - a U.S. No. 10 sieve is used to sort out larger invertebrates (e.g., mussels, burrowing mayflies, dragonfly larvae) from silt, sand and fine gravel by scooping the substrate to a depth of ca. 5 cm. A variety of collection sites are sampled in order to obtain 3 replicates in each substrate type (silt, sand and fine gravel).
2) *Netting* (optional) - a fine-mesh Surber sampler or a fine-mesh bag (300 µm) is used to collect sand and silt depositional areas by placing the net on the substrate and vigorously stirring the sediments in front of the net. An area of 0.1 m^2 is sampled for each replicate making sure, where possible, that replicates are taken from different depositional areas. The material is elutriated and sieved in a 300 µm nitex sampler constructed of PVC or other fine-meshed net.

c) Aufwuchs sample - small invertebrates associated with this habitat are obtained by washing a small amount of rocks, sticks, leaves, filamentous algae and moss into a medium-sized bucket half filled with water. The material is then elutriated and sieved with the nitex sampler.

d) Rock Picking - invertebrates are picked from 15 rocks (large cobble-small boulder size; 5 each from riffle, run and pool). Selected rocks are washed in a bucket half filled with water, then carefully inspected to remove invertebrates with fine-tipped forceps.

e) *Wood Sample* - pieces of submerged wood, adding ranging from roughly 3 to 6 meters (10 to 20 linear feet) and ranging from 5–15 cm (2–6 inches) in diameter, are individually rinsed into the wash-bucket. Pieces of wood are inspected for burrowers and crevice dwellers. Large diameter, well-aged logs should be inspected and hand-picked with fine-tipped forceps.

3. Alternative Methods

The following are methods that may be used for special studies, as approved by the WQB of KDOW.

a) Modified Traveling-Kick Method (TKM) - this is an adaptation of Hornig and Pollard's (1978) method. Three TKMs are taken on a transect across the stream at mid-riffle in the thalweg. In the event that the TKMs cannot be taken on a transect,

they will be collected diagonally in an upstream direction. A triangular or D-frame dipnet is placed on small cobble sizes or smaller sized substrate and moved in an upstream direction disturbing an area in front of the dipnet of 30 cm (1 ft). The distance covered in the Interior Plateau portion of the state is 1.5 m (5 ft) in 30 seconds, while the distance in the Eastern and Western Coal Fields and Jackson Purchase area is 3 m (10 ft) in one minute.

b) Surber Sampler - this sampler should be employed only when the riffles are shallow (20 cm or less) and the current is moderate. Three to four Surbers are taken on a transect across the stream at mid-riffle in the thalweg by methods outlined in Needham and Needham (1962) and Klemm et al. (1990). In the event that the Surbers cannot be taken on a transect, they will be collected diagonally in an upstream direction.

c) Modified Hester-Dendy Multiplate Sampler - three multiplates, as described by Fullner (1971), are attached by wire or cord to a flotation device and allowed to float 25–30 cm (1 ft) below the surface. The multiplates are collected at the end of three weeks during the summer in the Interior Plateau or six weeks in the spring or fall in the Interior Plateau and for six weeks in the rest of the state regardless of season.

d) Basket Sampler - these samplers, described by Mason et al. (1967), may be used in lieu of multiplates. The basket samplers should be filled with limestone rock of approximately 7.5 cm (3 in) in diameter or porcelain spheres of approximately the same diameter. Residence time is the same as the multiplates.

e) Drift Nets - these should be used in streams that have a current velocity of more than 0.5 m/sec (1.5 ft/sec). A minimum of two drift nets per station should be used. One net should be set 25 cm (10 in) from the surface of the river and one set 10 cm (4 in) below the surface of the water. The collection period should be from one to three hours. Data should be reported in number of organisms per 100 m^3 of flow. Additional information can be found in Klemm et al. (1990).

f) ***Dredges*** - these may be used in lentic-type environments in water depths greater than 1 m and collected on a transect. All dredge samples are to be washed in a 600 µm mesh bucket.

g) Nylon rope ladder - for freshwater mussel monitoring. This method was developed specifically to observe mussel populations or individual mussels in wadeable streams. It is used for long-term monitoring purposes. A rope ladder is constructed to form successive square-meter blocks, using ½ inch nylon rope and ¼ inch metal bars as rungs. Concrete rebar (½ X 36 in.) stakes driven downward into the stream banks serve as a permanent reference and attachment site for the ladder. The square meter blocks are numbered from left to right facing upstream. Mussel(s) located within each block are charted on paper to represent their location in the streambed.

Table 8-1. Summary of sampling methods for wadeable, moderate/high-gradient streams.			
Technique	**Sampling Device**	**Habitat**	**Replicates (composited)**
1m^2 Kicknet*	Kick Seine/Mesh Bucket	Riffle	4- 0.25m^2
Sweep Sample	Dipnet/Mesh Bucket	All Applicable	
Undercut Banks/Roots	"	"	3
Emergent Vegetation	"	"	3
Bedrock/Slabrock	"	"	3
Justicia beds	"	"	3
Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
Silt,Sand, Fine Gravel		Margins	
Coarse Seive	US No. 10 Seive		3
Fine Mesh	300 µm nitex net		3
Aufwuchs Sample	300 µm nitex net	Riffle-Run-Pool	3
Rock Pick	Forceps	Riffle-Run-Pool	15 rocks (5-5-5)
Wood Sample	Mesh Bucket	Riffle-Run-Pool	3-6 linear m

*Sample contents kept separate from other habitats

B. Wadeable Low-Gradient

Low-gradient streams are defined as streams that have velocities less than 0.013 m/sec (0.5 ft/sec) and naturally lack riffle habitat. The most productive habitats of these streams are typically woody snags, undercut banks and aquatic vegetation. A proportional sampling technique is used in most streams of the western parts of the state, particularly in ecoregions 72, 73 and 74. The method follows, in part, the Mid-Atlantic Coastal Plain Streams Workgroup (MACS) protocol (US EPA 1997a), which is also described in Barbour et al. (1999). Essentially, the technique is considered "proportional sampling," in which some predetermined number of sample units (20 in this case) is allocated among distinct and productive mesohabitats in relation to their proportion found within a 100 m stream reach.

A sample unit is called a "jab" in which a D- or A-frame net is thrust into the targeted habitat in a jabbing motion for approximately 0.5 m and then swept with the net 2 or 3 times to collect the dislodged organisms. For example, in a 100 m stream reach, if woody snags make up roughly 50% of the reach, submerged root mats 25%, and submerged macrophytes 25%, then 10 jabs are to be allocated to the snags, 5 jabs allocated to the root mats, and the last 5 jabs would be allocated to the macrophytes. If a jab becomes heavily clogged with debris and sediment, discard and repeat the jab. ***All material is composited*** into a wash bucket for further processing. Large leaves and twigs can be washed, inspected, and discarded to reduce the volume of the debris in the sample. Sand and sediment can be elutriated using a bucket and 600 µm sieve.

C. Headwater High-Gradient

In these small streams, riffle habitat predominates and is the primary targeted habitat. Benthic invertebrates should be collected in the spring index period (mid-February to early June) as this period offers the highest potential for macroinvertebrate diversity and abundance in these headwater streams. A collection consists of a composited semi-quantitative riffle sample and a composited

multi-habitat sample. ***These two sample types must be kept separate for a more effective diagnosis of impairment***. A summary of these collection techniques is shown in Table 8-2.

1. **Riffle Sample**
   For the semi-quantitative sample, invertebrates are collected from four 0.25 m^2 quadrat kicknet samples stratified within the thalweg of cobble-boulder riffle habitat. This habitat is targeted to ensure the highest species richness and abundance of macroinvertebrates. The thalweg of a riffle also guarantees the most flow permanence and substrate stability in these often intermittent streams. Two kicknet samples are allocated to each of two distinct riffles that are separated by at least one pool or run. This is done to help reduce between-riffle variability. The four samples are composited into a 600 µm mesh wash bucket to yield a 1 m^2 semi-quantitative sample. The composited sample is partially field processed using a U.S. #30 sieve (600 µm) and wash bucket. Large stones, leaves and sticks are individually rinsed and inspected for organisms and then discarded. Small stones and sediment are removed by elutriation using the wash bucket and U.S. #30 sieve.

2. **Multi-Habitat Sample**
   The multi-habitat, qualitative sample consists of a composite of 3 leafpacks, 3 jabs in sticks/wood, 3 jabs in soft sediments, 3 jabs into undercut banks/submerged roots, handpicking of 5 small pool boulders and approximately 2 linear feet of large woody debris. These techniques were described above in the wadeable Moderate/High-Gradient section.

Table 8-2. Summary of sampling methods for headwater, moderate/high-gradient streams.

Technique	Sampling Device	Habitat	Replicates (composited)
1m^2 Kicknet*	Kick Seine/Mesh Bucket	Riffle	4-0.25m^2
Sweep Sample	Dipnet/Mesh Bucket	All Applicable	
Undercut Banks/Roots	Dipnet/Mesh Bucket		3
Sticks/Wood			3
Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
Silt,Sand, Fine Gravel	Dipnet/Mesh Bucket	Margins	3
Rock Pick	Forceps	Pool	5 sm. boulders
Wood Sample	Forceps/Mesh Bucket	Riffle-Run-Pool	2 linear m

* Sample contents kept separate from other habitats

D. Non-wadeable Streams

Samples taken from non-wadeable (boatable) streams will utilize methods similar to those outlined for wadeable low-gradient streams (section 2.B.). A site will consist of a 300-meter stream reach. The proportional sampling technique, utilizing the 20 "jab" method discussed in Section 2B will be augmented with dredge samples, rock picking and a wood sample. Three dredge samples, taken with a petite ponar grab sampler, are taken from each of three transects; one dredge sample is taken from

mid-stream and one each from the right and left bank. The sample material is washed thoroughly in a 600 µm mesh wash bucket, then the macroinvertebrates are removed and stored in 70% ethanol. Where available, pick 15 rocks (large cobble-small boulder) and wash and pick 6 m of wood (5–15 cm in diameter).

E. Probabilistic (Random) Bioassessments

Probabilistic bioassessments incorporate most of the sampling protocols for macroinvertebrates described above. The stream reach to be assessed is defined as 40Xs the width of the stream. In sampling each stream, the random design restricts the investigator to available habitat offered at each reach. If the stream is a high-to moderate-gradient stream and the particular reach contains no riffle-run habitat (a rare occurrence), the macroinvertebrates will be collected using the 20-jab proportional sampling technique (see Section 2.B.). This method is employed routinely in coastal and low-gradient streams where riffle-run habitat does not occur. Otherwise, a kicknet is used to collect macroinvertebrates from the riffle habitat and a D- or A-frame dipnet is employed to collect macroinvertebrates from other habitats that may be available, such as roots/undercut banks, woody debris, aquatic vegetation and leaf packs. Collected benthos are partially field processed and preserved in 95% ethyl alcohol. Those organisms collected from the riffle habitat are kept separate from those collected in other habitats.

III. SAMPLE PROCESSING

A. Sorting

In the field, large sticks and leaves are washed, inspected for organisms and discarded. Rocks should be elutriated and hand washed with a bucket and 600 µm sieve. This process is repeated until a manageable amount of debris and organisms (relative to size of sample container) can be preserved for laboratory sorting. Samples may be partially field picked in the field using a white pan and fine-tipped forceps. Club soda may be used to retard the movement of aquatic organisms when field sorting. Approximately a tablespoon of material to be sorted is placed in a white pan with a small amount of water. The material is dispersed evenly throughout the pan, and all macroinvertebrates are removed and placed in 70% ethanol.

Sorting in the laboratory is done with the aid of a circline lamp or dissecting scope against a white background. Staining invertebrates with either rose bengal or phloxine B at a concentration of 100 g/L of ethanol may be done to aid in sorting operations. Sugar floatation techniques may also be employed to facilitate sorting. While entire picks are routinely done at KDOW, unmanageable amounts of organisms and debris may be proportionally subsampled by dividing all of the material into $1/4^{th}$s, randomly choose one of the quarters and remove all organisms. If at least 300 organisms are not obtained from the subsample, pick additional quarters completely until the target number is reached. After subsampling, scan the remaining sample under low magnification for rare or large organisms not found in the original subsample. In addition, certain taxa (e.g., chironomids, hydropsychid caddisflies) may be subsampled (10%, 20% or 25%) using gridded pans and a random numbers table.

B. Preservation

Samples are initially preserved in the field in 95% ethanol. Upon returning to the laboratory, all sorted samples are transferred to a fresh 70% ethanol solution.

C. Labeling

While at the sampling location, all macroinvertebrate samples will receive a label. The label may be placed in the sample jar or written directly on some portion of the jar. The label will include the site number, if known, stream name, location, county, date sampled and the collector's initials. A permanent label will be placed in the collection jar either when the sample is returned to the laboratory, or when it is identified. This label will include the site number, stream name, county, date sampled, latitude, longitude, mile point and collector names.

D. Taxonomy

All taxonomic identifications are made to the lowest practical level, using the most current taxonomic references available. A partial list of taxonomic references used by KDOW is found at the end of this chapter. A current master taxa list is found in Appendix D-1.

IV. DATA ANALYSIS

The use of multiple community attributes to assess instream biological impairment has become widely accepted. This approach was first developed by Karr (1981) for midwest fish communities and now has been refined regionally and used throughout the United States. Karr's methods involved using a variety of community attributes, referred to as "metrics," to assess the condition of biological communities. Each metric is expected to contribute pertinent ecological information about the community under study. Examples of the application of the metric approach to aquatic macroinvertebrate communities can be found in Nuzzo (1986), Ohio Environmental Protection Agency (1987), Bode (1988), Shackelford (1988), Plafkin et al. (1989) and Barbour et al. (1999).

A. Core Metrics

The KDOW's metric selection process uses statistical properties of redundancy and sensitivity to evaluate the power of metrics that can discriminate between impaired and unimpaired sites. Metric scoring criteria are established using percentiles of the reference and non-reference data distribution. The following is a list and explanation of each metric commonly used by KDOW. Note that metric combinations and scoring criteria may vary among ecoregions and stream sizes. [**Many of these metrics (e.g., mHBI, % composition metrics) require quantitative or semi-quantitative sampling (i.e., calculated from riffle kicknet sample, or 20-jab technique.**]

1. **Taxa Richness**. This refers to the total number of distinct taxa present in the composited sample (both semi-quantitative and qualitative samples combined). In general, increasing taxa richness reflects increasing water quality, habitat diversity and/or habitat suitability.

2. **Ephemeroptera, Plecoptera, Trichoptera Richness (EPT).** This is the total number of distinct taxa (both semi-quantitative and qualitative samples combined) within the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera found in the composited sample. This index value will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

3. **Modified Hilsenhoff Biotic Index (mHBI)**. The HBI was developed to summarize the overall pollution tolerance of a benthic arthropod community with a single value (Klemm et al. 1990). Hilsenhoff (1977), using a range of 0-5, originally developed the index for Wisconsin riffle/run streams experiencing organic pollution. Hilsenhoff (1982, 1987) later refined the index, expanding the scale to range from 0 to 10. Plafkin et al. (1989) modified the index to include non-arthropod benthic macroinvertebrates. Hilsenhoff (1987) developed tolerance values for a variety of macroinvertebrates from Wisconsin, and Plafkin et al. (1989) added additional tolerance values. However, KDOW uses tolerance values developed by North Carolina Division of Environmental Management (NCDEM) (Lenat 1993) as well as values developed from KDOW data. These HBI values have been regionally modified for streams of the southeastern United States. Both Hilsenhoff (1988) and NCDEM have developed seasonal correction factors for the HBI. Several states, including Kentucky, have used the mHBI to assess impacts other than organic enrichment and found the mHBI to be a valuable metric. An increasing mHBI value indicates decreasing water quality. The formula for the HBI is as follows:

$$m\,HBI = \frac{\sum n_i \; x \; a_i}{N}$$

where:
n_i = number of individuals within a species **(maximum of 25)**,
a_i = tolerance value of the species,
N = total number of organisms in the sample **(adjusted for $n_i \geq 25$)**.

4. **Modified Percent EPT Abundance (m%EPT).** This metric measures the abundance of the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera. The relatively tolerant and ubiquitous caddisfly *Cheumatopsyhce* is excluded from the calculation. Increasing values indicate increasing water quality and/or habitat conditions.

5. **Percent Ephemeroptera (%Ephem).** The relative abundance of mayflies is calculated to show impacts of metals and high conductivity associated with mining and oil well impacts. Ephemeroptera abundance normally declines in the presence of brine and metal contamination.

6. **Percent Chironomidae+Oligochaeta (%Chir+%Olig).** This metric measures the relative abundance of these generally pollution tolerant organisms. Increasing abundance of these groups suggests decreasing water quality conditions.

7. **Percent Primary Clingers (%Clingers).** This habit metric measures the relative abundance of those organisms that need hard, silt-free substrates to "cling" to. Merritt and Cummins (1996) and Barbour et al. (1999) list habits for most insect genera. Habit information for non-insect taxa can be determined from Pennak (1989), Thorp and Covich (1991) and Barbour et al. (1999).

B. Supplemental Metrics

These metrics may be used in special situations such as intensive surveys and nonpoint source studies that evaluate a particular impact. Functional feeding group abundances may also be calculated to provide insight into food/energy relationships among sites.

1. **Jaccard Coefficient of Community Similarity**. This similarity index measures the degree of taxonomic similarity between two stations in terms of taxon presence or absence. Coefficient values, ranging from 0 to 1.0, increase as the similarity with the reference station increases. The formula is as follows:

$$Jaccard\ Coefficient = \frac{c}{a+b+c}$$

where:
a = number of taxa in sample A but not B
b = number of taxa in sample B but not A
c = number of taxa common to both samples
Sample A = reference station
Sample B = station of comparison

2. **Percent Community Similarity (PSc)**. The PSc index, discussed by Whittaker (1952), was used by Whittaker and Fairbanks (1958) to compare planktonic copepod communities. It is a good index in bioassessments because it shows community similarities based on relative abundance, thus, giving more weight to dominate taxa than rare ones. Percent community similarity values range from 0 (no similarity) to 100 percent. The formula for calculating PSc is:

$$PSc = 100-0.5\Sigma(a-b) = \Sigma min(a,b)$$

Where:
a = percentage of taxa a in sample a
b = percentage of taxa b in sample b

3. **Percent Contribution of Dominant Taxa (PCD_5).** This simple measure of redundancy and evenness adds the relative abundance percentages of the five dominant taxa. Highly redundant communities (i.e., communities highly dominated by a few taxa) may reflect a degraded condition.

4. **EPT/Chironomidae (Ratio of EPT to Chironomidae Abundances)**. This metric uses the ratio of these indicator groups as a measure of community balance. Communities with a good biotic condition would be expected to have a substantial representation of EPT taxa. Skewed populations having a disproportionate number of generally tolerant chironomids relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987).

5. **Dominants in Common, Five (DIC_5)**. This metric, developed by Shackleford (1988), measures the similarity of a reference station and a station of comparison based on the five most abundant taxa at each station. The DIC_5 provides a measurement of substitution between the reference community and the downstream station.

6. **Dominants in Common, Ten (DIC_{10})**. This metric, also developed by Shackleford (1988), is the same as the DIC_5 metric, but is based on the ten most abundant taxa at each station.

7. **Percentage of *Cricotopus*+*Chironomus* Abundance to Total Chironomidae (Cr+Ch /Chironomidae)**. This measures the abundance of the pollution-tolerant genera *Cricotopus* and *Chironomus* to the total abundance of the family Chironomidae.

Additional information on various metrics may be found in Plafkin et al. (1989), Klemm et al. (1990), Resh and Jackson (1993) and Barbour et al. (1992, 1999). The functional feeding group concept is discussed by Cummins (1973), and genus-level functional feeding group designations for aquatic insects are provided by Merritt and Cummins (1996).

C. Macroinvertebrate Bioassessment Index

Macroinvertebrate data analysis for wadeable or headwater streams is accomplished by using the multimetric approach. Metric criteria are based on reference reach data and may vary from region to region and with stream size (i.e., wadeable or headwater). Core metrics in wadeable streams are

Taxa Richness, modified Hilsenhoff Biotic Index, EPT Richness, modified %EPT Abundance and %Chironomidae+Oligochaeta Abundance and % Clinger Abundance. Additionally, supplemental metrics are used depending on type of impact, stream size, subecoregion, etc. Each metric is given a calculated score (range 0–100) based on the percent of the standard metric value (i.e., the 95th percentile or 5th percentile). These percentile thresholds are used to eliminate outliers. The formulae for calculating MBI scores are shown in Table 8–3. The individual metric scores are summed and then averaged to produce a Macroinvertebrate Bioassessment Index (MBI) value. Thresholds are established to assign narrative water-quality ranking of Excellent, Good, Fair, Poor and Very Poor. The KDOW is in the process of calibrating, or refining, metric scoring criteria for ecoregions and stream sizes. A separate document detailing the metric selection and evaluation process and bioassessment interpretation is currently in preparation. Contact the Ecological Support Section of the WQB for current 95th %ile and 5th %ile values.

Table 8-3. Examples of metric scoring formulae for the Macroinvertebrate Bioassessment Index.

Metric	Formula
TR	$\frac{TR}{95th\%ile} X100$
EPT	$\frac{EPT}{95th\%ile} X100$
MHBI	$\frac{10 - mHBI}{10 - 5th\%ile} X100$
m% EPT	$\frac{m\%EPT}{95th\%ile} X100$
%Clingers	$\frac{\%Clingers}{95th\%ile} X100$
% Chir+Olig	$\frac{100 - \%Chir + Olig}{100 - 5th\%ile} X100$

In probabilistic (random) sampling, the MBI may not be representative in cases when atypical habitat is sampled. For example, in streams where riffle-run habitat typically occurs but by chance this habitat did not occur in the randomly chosen stream reach, the sampling techniques and the habitat assessment must be considered in making the final aquatic life use determination. Otherwise, the evaluation is made solely using the MBI.

D. Quality Assurance/Quality Control (QA/QC)

The following is a list of QA/QC procedures that will be followed for the collection, sorting and identification of macroinvertebrate samples. All personnel involved in the collection and identification of macroinvertebrates will have prior educational qualifications as well as appropriate in-house training.

1. **Sample Collection**. Five percent of samples collected by KDOW will be duplicated to evaluate precision and repeatability of the technique and/or the sampling crew. The field crew will select a stream reach that has sufficient habitat to accommodate a duplicate sample. After the duplicate sample has been taken, with the appropriate sampling methods for that stream reach, it will be labeled as the duplicate and processed with the rest of the samples. After any sampling has been completed, all sampling gear (nets, sieves, buckets, etc.) will be thoroughly cleaned to remove all macroinvertebrates so that specimens are not carried over to the next site. The equipment should be examined just prior to sampling the next site to insure that no macroinvertebrates are carried over.

2. **Laboratory Sorting**. Ten percent of the pans sorted will be examined by a second qualified biologist to insure that all organisms are being removed from the sorting pan or grid. If less than 10 organisms are found in the pan or grid, the sample is considered passed. If more than 10 organisms are found, then the sample fails and another successive pan will be checked. This will continue until the sorter passes the procedure. The sorting pans will be thoroughly rinsed after each pan is picked.

3. **Identification**. Five percent of samples collected by KDOW will be re-identified by a second taxonomist to evaluate precision and accuracy. A target of 90% similarity in taxonomic composition is acceptable. A third taxonomist will reconcile differences in identifications between the two taxonomists, if necessary. A voucher collection of macroinvertebrates will be maintained by the WQB of KDOW. If possible, three to five specimens of the vouchered taxon will be placed in the vial. These specimens will be properly labeled, preserved and stored in the WQB laboratory for future reference. Rare and/or difficult taxa will be verified by other WQB biologists or noted taxonomic experts.

    a) A macroinvertebrate bench sheet (Appendix D-2) shall be produced during the sample identification process. After the sample has been identified, the data will be entered into the WQB database.

    b) When available, KDOW biologists will take part in suitable macroinvertebrate identification training (offered by U.S. EPA, state universities and other groups) as this will help ensure consistent and accurate macroinvertebrate identification. In addition, the WQB will maintain a library and list of taxonomic references.

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9 - FISH

FISH COMMUNITY STRUCTURE

I. INTRODUCTION

The evaluation of fish community structure is an important component of biological monitoring, intensive surveys, Exceptional Water determinations and reference reach surveys, all of which provide reliable assessments for the Clean Water Act (CWA) Section 305b. The primary goal of evaluating fish community structure to ensure accurate assessments for the CWA is to perform a fish Index of Biotic Integrity (IBI) on the sampling event. Advantages of using fish as biological indicators include their widespread distribution from small streams to all but the most polluted waters, their utilization of a variety of trophic levels, their stable populations during summer months and the availability of extensive life history information (Karr et al. 1986). The methods used for collecting, analyzing and reporting of fish community structure data are outlined in this section.

II. SAMPLING METHODS

Standardization of methods is essential since it will allow streams to be assessed and compared, therefore, establishing trends and long-term monitoring (Bonar and Hubert 2001). To ensure collection of standardized fish community data, stream size (i.e., drainage area) has been used to designate streams to two classes, headwater and wadeable, and a set methodology is outlined for each classification. For fish assessments, headwater streams are streams with a drainage area less than 12 mi.^{2} (30.1 km^2). Wadeable streams are streams with a drainage area greater than 12 mi.^{2} (30.1 km^2). However, streams between the drainages of 8-12 mi.^{2} (20.7-30.1 km^2) fall within a "gray" area of stream classification and best professional judgment should be used to determine if the stream is headwater or wadeable.

The sampling index period is **mid-March through October**. Prior to sampling, the field crew should research the distributional records and familiarize themselves with the possible ichthyofauna for a particular basin and compile a species list for that basin. Special awareness and precautions should be made for the possibility of any Federal Threatened/Endangered Species encountered.

A. Headwaters

The sample reach must range between 100-125 meters in length, which should consist of riffles, runs and pools if they are present. Also, the reach must not be associated within the immediate area (<150 meters) of major tributary confluences or human structural influences, such as bridges, road crossings (fords), low head dams or any other similar structure, unless the purpose of obtaining the fish community data is related to these influences. Sampling in headwater streams must consist of using a backpack electrofisher unit working in an upstream manner (**collectors must be familiar with and follow all safety procedures as suggested by the manufacturer**). The electrofishing duration within the sample reach should be a minimum of 600 "shocking" seconds and a maximum of 1000 "shocking" seconds.

The lower and upper ends of the reach should be associated with a natural instream barrier such as a riffle. The sampling crew should consist of a minimum of two members with at least one member having prior electrofishing experience. The crew should work upstream shocking in a side-to-side/bank-to-bank sweeping technique. The crew collects the stunned fish with dip nets and places them into a livewell/bucket for preservation or identification after the reach has been sampled. One pass of the reach is sampled from the downstream end to the upstream end with all recognizable habitats thoroughly sampled, following the sweeping technique (Barbour et al. 1999). In streams with larger pool habitat, a seine should be used to sample the area more efficiently. The experience of the crew and their ability to see and net the fish improves the effectiveness of sampling the reach. Polarized sunglasses are optional, since they will cut down on the glare of the water. Also, physical features such as water clarity, flow and depth and time of day need to be considered to obtain optimal success in sampling. If proper conditions are not present sampling needs to be postponed until an efficient sampling effort can be made.

B. Wadeable

Wadeable streams are larger bodies of water and provide more variability of habitat than headwater streams, so the reach sampled will be determined by a greater time designation within the given length designation. Because of that variability, a combination of seining and electrofishing is used, since both methods have advantages and disadvantages in habitat types and species groups. The combination of seining and electrofishing yields better results than with one technique independently (Onorato et al. 1998; Yoder and Smith 1999). The collectors should be aware of the advantages and shortcomings of each technique. KDOW has observed electrofishing to be more effective in streams that may have numerous boulders and/or undercut banks and/or woody debris. KDOW also has observed that electrofishing tends to be biased toward catostomid and centrarchid members while not fully representing the schools of cyprinids in large pools. However, cyprinids can be effectively sampled with a seine in large pool habitats to yield a better representation of their presence in the community (Onorato et al. 1998).

The reach length should be a minimum of 100 meters and should not exceed 200 meters (documentation of the sample reach should be noted). The sample reach should consist of at least two riffles, runs and pools each. In cases where two riffles, runs and pools cannot be sampled, either one riffle, run and pool is sampled or the recommended reach length of the stream is sampled. Sampling must be done using a seine (3.4 × 1.8 m with 0.3 cm mesh or any other suitable size) and an electrofisher (any suitable generator or battery powered electrofisher may be used). A seine is used for approximately 30-60 minutes from start to finish. The electrofisher (backpack, tote barge or similar units) is typically used in areas not efficiently sampled with the seine (e.g., root masses, undercut banks, rock slabs, boulder/cobble substrates, fallen trees, etc). The electrofisher should be used for at least 600 “shocking” seconds to a maximum of 1800 “shocking” seconds of effort. Also, as in headwater streams, the electrofishing method should be done in a sweeping manner, and the reach must not be associated within the immediate area (<150 meters) of major tributary confluences or human structural influences. Best professional judgment by the collector is used to determine the appropriate gear and effort for a particular habitat, but a combination of the two methods (seine and electrofisher) must be used. Typically a reach is thoroughly sampled when no new species are being collected and all habitats have been sampled.

Documentation of methodology is required to help provide insight into the results of the sampling effort, particularly if only one technique is used. Again the efficiency of sampling depends on the experience of the crew and their ability to see and net the fish. Polarized sunglasses are optional. The water clarity, flow and depth and the time of day need to be considered to obtain optimal success in sampling. If these conditions are not adequate and/or practical, sampling needs to be postponed until an efficient sampling effort can be made.

C. Preservation

Fish collections are preserved in the field with a 10%-15% buffered formalin solution. Large specimens are to be identified in the field, recorded and released, unless the specimen(s) represent a significant ichthyological find (e.g., state or drainage record), then they are to be preserved as voucher specimens. Easily identified fish that are collected in large numbers (i.e., *Campostoma* spp.) are also recorded in the field and released. Photographs of large specimens and/or vouchers of all released specimens need to be made for verification. If possible, at least five specimens of each species released should be kept as vouchers from the sample event. If a species or genus is viewed but not collected and if positively identified, these records should be noted (i.e., *Hypentelium nigricans*, *Micropterus* spp. or *Lepomis* spp.).

III. LABORATORY PROCESSING

Fix fish in formalin for at least 2 weeks (larger fish should be fixed for at least 3–4 weeks unless injected with a formalin solution in the field). Samples are then rinsed and soaked for 1–2 days in tapwater. Transfer fish to 70% ethanol solution for long-term preservation and storage. Identify and count fish using all available taxonomic keys and distribution records. In a given sampling season, 10% of the samples collected should be re-identified by another qualified/trained fish taxonomist for the purpose of quality assurance/quality control. Independent taxonomic verifications of selected species are obtained, when needed, from recognized experts (e.g., Ecological Support Section KDOW or Kentucky State Nature Preserves Commission).

Species and number of individuals collected are recorded on data sheets (Appendix E-1) and used for evaluating species composition, relative abundance, species richness and presence/absence of indicator species. The occurrence of species that are listed as rare, endangered or threatened by federal or state agencies is also documented, and the appropriate agencies are notified with the Threatened/Endangered Species Report Form (Appendix E-2). Other characteristics of fish communities that are evaluated, as needed, include the presence of hybrids, size/age distribution of populations, incidence of disease and occurrence of parasites. These and other non-routine assessments of fish communities are included in specific study plans.

IV. QUALITY ASSURANCE AND QUALITY CONTROL

A. Quality Assurance and Quality Control in the field (Barbour et al. 1999):

A field crew will consist of at least one trained ichthyologist. The ichthyologist is to make sure voucher representatives (5 specimens, if possible) of all fish are taken, and all specimens which are in question of identification are preserved correctly for laboratory examination. All released

specimens will be noted. Collection labels are to be accurate and complete with all pertinent information that was previously discussed.

In a sampling season 5% of the samples will be duplicated/replicated by a field crew. The duplicate/replicate samples will be taken either at the exact sample site within a two-week period or taken on the same day within the sample reach by either the same field crew or an alternate crew to ensure precision and repeatability of the sampling technique.

B. Quality Assurance and Quality Control in the laboratory (Barbour et al. 1999)

All samples will be preserved correctly to ensure optimal condition of specimens. Label information must be accurate and complete. The ichthyologist will be trained and up to date with the current identification and nomenclature of Kentucky fishes. The use of all pertinent keys and distributional records (see taxonomic references) is required and final identification will be cross-referenced with the KDOW voucher collection. Ichthyologists should discuss any problem identifications with other in-house ichthyologists. Recognized taxonomic experts will assist in difficult specimens and confirmation of specimens. Ichthyologists will participate in fish identification workshops, seminars and training sessions when opportunities arise.

In a sampling season 10% of the collections will be re-identified and enumerated by another ichthyologist. The re-identification and enumeration of a collection should exceed 90% accuracy. Duplicate samples should exceed 75% similarity (Whittaker 1952). Duplicate/replicate samples will be processed and analyzed in the same procedures as regular samples.

V. DATA ANALYSIS

A. Index of Biotic Integrity

The Index of Biotic Integrity (IBI) as described by Karr (1981) was used to assess the fish community structure and biotic integrity of midwestern streams. This IBI was composed of 12 equally weighted metrics that were grouped into three general categories: Species Richness and Composition (Category I), Trophic Composition (Category II), and Fish Abundance and Condition (Category III). Each metric was assigned a 5, 3 or 1 value depending upon whether the obtained value strongly approximates the expected value (5), somewhat approximates the expected value (3) or does not approximate the expected value (1). The individual metric scores were summed and a total IBI score ranging from 12–60 was achieved. Metrics in Category I often vary with region and stream size, while less variation was usually found among those in Categories II and III (Karr et al. 1986). Five classifications based on total IBI scores were assigned by Karr (1981) to describe the quality of the fish community at each site. Scores that fall between categories were evaluated based on professional experience.

While the theory of using a multimetric fish index to assess stream health has sustained and prospered over time, numerous modifications and testing of the original IBI have been made to provide more accuracy and precision within a particular region and/or water body type (Ohio 1987, Minns et al. 1994; Barbour et al. 1999, Hughes and Oberdorff 1999, Smogor and Angermeier 2001). In the early 1990s KDOW started a Reference Reach Program to document

natural patterns in the biota and to develop biocriteria among the Commonwealth's lotic systems (KDOW 1997). However, gaps exist in the state database; therefore, further collection and data analysis is needed. Given the existing data and following recent IBI framework approaches (Barbour et al. 1999, Simon [ed.] 1999, McCormick et al. 2001, Smogor and Angermeier 2001), the IBI is being modified from the KDOW (1997) version to provide statewide coverage, easier application and better precision among users.

B. Metrics

Fish assemblages in the state have shown strong correlation with ecoregions, basins, physiographic regions and stream size. Development of criteria for an IBI must be region- and stream size-specific to correspond with the differences within the ecoregion/basin framework (Fausch et al. 1984, Angermeier et al. 2000). Therefore, several candidate metrics were tested and validated for their sensitivity and variation among each region and stream size following methodology found in Smogor and Angermeier (1999) and McCormick et al. (2001). Metrics were selected to demonstrate attributes of a fish community that show responsiveness to disturbances, predictability and uniformity throughout the state. A list of candidate metrics is provided in Table 9-1. The metric selection process uses statistical properties of redundancy and sensitivity to evaluate the discriminating power of a metric between impaired and relatively unimpaired sites (reference sites). After testing the candidate metrics, eight metrics were selected to structure the multimetric IBI. The following list is an explanation of each metric used in the Kentucky IBI. A master list of Kentucky fishes with their trophic and tolerance classifications is provided in Appendix E-3. The ecological classifications in the master taxa list has been compiled using scientific literature, historic data, consultation with fisheries biologists, and professional experience (Ohio EPA 1987, Etnier and Starnes 1993, KDOW 1997, Goldstein and Simon 1999, McCormick et al. 2001). Any necessary modifications to the list will be made as additional information becomes available

1. **Native Species Richness (NS)**. This is the total number of native species present in a sample. Native richness is expected to decline with impairment. Non-native species are excluded since they tend to invade with impairment. This metric will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

2. **Darter, Madtom and Sculpin Richness (DMS)**. This is the total number of the species in a sample within the tribe Etheostomatini (darters), the genus *Noturus* (madtoms) and the genus *Cottus* (sculpins). These groups are generally sensitive to pollution and are expected to decline with impairment. This metric will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

3. **Intolerant Species Richness (INT)**. This metric represents the total number of intolerant species collected in a sample. Intolerant species are expected to decline with impairment. This metric will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

4. **Water Column Species Richness (WC)**. This metric is a combination of four metrics. It is the total number of species, excluding tolerant members, from the family Cyprinidae along with the

Sunfish, Top Carnivore and Sucker metrics used in KDOW (1997). This metric is expected to decline with impairment. This metric will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

5. **Simple Lithophilic Spawning Species Richness (SL)**. This metric is the total number of simple lithophilic spawning species. This metric represents species that require relatively clean gravel and exhibit simple spawning behavior (Ohio 1987, Simon 1991). The metric is considered a habitat metric and is expected to decline with impairment and be particularly sensitive to siltation (Berkman and Rabeni 1987).

6. **Proportion of Insectivorous Individuals (% INST)**. This metric is the relative abundance of insectivorous individuals excluding tolerant individuals. This metric represents a proportion of individuals that are fairly sensitive and feed primarily on insects. The metric is expected to decline with impairment. This metric will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

7. **Proportion of Omnivorous Individuals (% OMNI)**. This metric is the relative abundance of omnivorous individuals. This metric represents a proportion of individuals that are considered generalist in their feeding behavior. This metric is expected to increase with impairment. This metric will usually increase with decreasing water quality, habitat diversity and/or habitat suitability.

8. **Proportion of Tolerant Individuals (% TOL)**. This metric is the relative abundance of tolerant individuals. This metric is to represent a proportion of individuals that are pollution tolerant and invade with impairment. Therefore, this metric is expected to increase with impairment. This metric will usually increase with decreasing water quality, habitat diversity and/or habitat suitability.

Table 9-1: Candidate Metrics for the Index of Biotic Integrity (IBI)[1]

Metrics	Description	Response
Total Number of Species	Measures the number of species	Decreases
Total Number of Native Species	Measures the number of native species	Decreases
Number of DMS Species	Measures the number of Darter, Madtom and Sculpin Species	Decreases
Number of Darter Species	Measures the number of Darter Species	Decreases
Number of Sunfish Species	Measures the number of Sunfish Species	Decreases
Number of Intolerant Species	Measures the number of Intolerant Species	Decreases
Number of Cyprinid Species	Measures the number of Minnow Species	Decreases
Number of Sucker Species	Measures the number of Sucker Species	Decreases
Number of Simple Lithophilic Spawning Species	Measures the number of Simple Lithophilic Spawning Species	Decreases
Number of Top Carnivores	Measures the number of Top Carnivores Species	Decreases
Percent Tolerant Species	Relative abundance of tolerant individuals	Increase

Table 9-1: Candidate Metrics for the Index of Biotic Integrity (IBI)[1]		
Metrics	**Description**	**Response**
Percent Omnivorous Species	Relative abundance of individuals as generalist feeders	Decreases
Percent Insectivorous Species	Relative abundance of individuals as insect dominant Feeders	Decreases
Percent Darter Individuals	Relative abundance of darter individuals	Decreases
Number of Tolerant Species	Measures the number of Tolerant Species	Increases
Percent Green Sunfish	Relative abundance of Green Sunfish individuals	Increases
Total Number of Individuals	Measures the number of individuals collected (catch/effort)	Decreases
Percent Top carnivores	Relative abundance of individuals as top carnivores	Decreases
Percent Dace Individuals	Relative abundance of *Clinostomus*, *Phoxinus* and *Rhinichthys*	Decreases
Number of Darter and Sculpin Species	Measures the number of Darter and Sculpin Species	Decreases
Number of Darter and Madtom Species	Measures the number of Darter and Madtom Species	Decreases
Number of Headwater Species	Measures the number of Headwater Species	Decreases
Percent Headwater Species	Relative abundance of Headwater Species	Decreases
Percent Insectivorous Cyprinids	Relative abundance of Insectivorous Cyprinid Individuals	Decreases
Percent Pioneering Species	Relative abundance of Pioneering individuals	Increases
Percent Simple Lithophilic Spawning Species	Relative abundance of Simple Lithophilic spawning Individuals	Decreases
Percent Hybrids	Relative abundance of hybrid individuals	Increases
Percent Intolerant Species	Relative abundance of intolerant individuals	Decreases
Percent Diseased Individuals	Relative abundance of individuals with diseases, deformities, tumors and/or fin and skeletal anomalies	Increases
Percent Stoneroller Individuals	Relative abundance of *Campostoma* individuals	Increases
Percent Suckers	Relative abundance of Round-bodied Sucker individuals	Decreases
Percent DMS Species	Relative abundance of Darter, Madtom and Sculpin individuals of the community	Decreases
Number of Water Column Species	Measures the number of cyprinids, sunfish, top carnivores and catostomids, excluding tolerant members	Decreases
Number of Pool Species	Measures the number of Cyprinidae, Catostomidae and Centrarchidae Species, excluding tolerant members	Decreases

[1] **Bolded Metrics from Karr (1981)**

Table 9-2: Metrics Used in the Kentucky IBI		
SPECIES RICHNESS AND COMPOSITION		**Response to Disturbances**
1.	Richness of native fish species (NS)	Negative
2.	Richness of darter, madtom, sculpin species (DMS)	Negative
3.	Richness of intolerant species (INT)	Negative
4.	Richness of water column species (WC)	Negative
TROPHIC COMPOSITION		
5.	Proportion of individuals as insectivores (% INST)	Positive
6.	Proportion of individuals as omnivores (% OMNI)	Negative
FISH ABUNDANCE AND CONDITION		
7.	Richness of simple lithophilic spawning species (SL)	Negative
8.	Proportion of individuals as tolerant species (% TOL)	Positive

C. IBI Criteria

Currently, criteria are being developed, calibrated for drainage area, updated and tested for the various regions in the state using the 8 metrics. Each metric is given a calculated score (range 0–100) based on the percent of the standard metric value (i.e., the 95th percentile or 5th percentile predicted from the reference database). These percentile thresholds are used to eliminate outliers. The individual metric scores are summed and then averaged to produce an Index of Biotic Integrity (IBI) score. Thresholds are established to assign narrative water-quality ranking of Excellent, Good, Fair, Poor and Very Poor. The KDOW is in the process of calibrating, or refining metric scoring criteria for ecoregions and stream sizes. A separate document detailing the metric selection, the evaluation process and the bioassessment interpretation is currently in preparation. When available, the criteria for the state and/or a particular region can be obtained from the Ecological Support Section upon request.

VI. REPORTING FISH DATA

A. Community Structure Data

The condition of the fish community and the related stream health is determined by calculating an IBI for the site. Relative abundance, species composition, richness, the evaluation of species tolerances to environmental perturbations and the condition of fishes are all attributes that are factored into the IBI score in the form of metrics. Shifts in these respective attributes within the community should indicate a change in the stream condition, positive or negative. In addition, the presence or absence of indicator species and the presence of rare or endangered species should provide insight into the stream condition and the causes for respective changes or condition. Correlations with habitat, landuse, water quality and any other pertinent information should be made to interpret the IBI score and to provide intuitive information to the stream ecosystem function and condition. The age and size class distribution of species populations should be noted and assessed in relation to recruitment potential; spawning and nursery area availability should also be considered. Any limiting factors, either natural or human-induced, are reported.

B. Distribution Data

Monitoring of the fish community structure is useful for stream health and fish populations as a whole, but may be too broad for the monitoring of a particular species. Therefore, reliable records from collections can provide useful status information (e.g., rare, common, etc.) for a given species in the state. Considerable data are available on fish distribution in Kentucky (Burr and Warren 1986, KDOW data) and environmental requirements of many of the more common species. Distribution is affected by many natural factors, including evolution of drainage patterns, stream order, substrate, temperature, cover availability, gradient, current velocity, seasonal flow variability and presence and abundance of food organisms (Maret 1999 and Strange 1999). Fish have inherent ranges of tolerances for many of these natural factors. A variation outside these ranges of tolerance may be the result of detrimental conditions that are reflected in the presence, structure and relative abundance of fish populations. Therefore, a compilation of all existing ichthyological data (both historic and present fish population data and field survey data) is necessary to assist in the management of a species or species groups. Also, physical and chemical characteristics of the stream are assessed in order to determine habitat availability and suitability for a species. All of these aspects help provide insight into the status and condition of a given species population.

C. Conclusions

Conclusions based on all existing data for a site are drawn, and recommendations or subsequent corrective actions are made, as applicable. Conclusions should take into account the relations of the surrounding land use, in-stream habitat (micro and macro), water chemistry, collecting conditions, watershed history and other suitable factors. Fish collection data may be compiled and published as a separate technical report as necessary.

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